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ERRATA

Page 60, legend to Plate A, line 4, "tugidity" should read "turgidity."
Page 62, Table I, column 6, line 3, under "Per cent," "48.3" should read "4.83."
Page 72, line 14, "standards deviations" should read "standard deviations."
Page 137, Table II, column 4, line 3, "69" should read "59."
Page 207, paragraph 3, line 10, "the specimens" should read "other specimens."
Page 252, legend to Plate 33, D, "×90" should read "×1,000."
Page 340, line 30, "Murreaa" should read "Murrea."
Page 358, Plates 50–51, the plate numbers should be interchanged.
Pages 373–376 (Tables iv–xi) and pages 381–384 (Tables xiv–xxi), the headings for the abscissae and the ordinates should be interchanged.
Page 610, lines 9 and 10, "This was the only point at which *O. humilis* had been established when the collections were made" should read "At this point only *O. humilis* had been established when the collections were made."
Page 611, lines 15 and 28, "*Gibberella* spp." should read "*Gibberella* sp."

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TRUE NATURE OF SPINACH-BLIGHT[1] AND RELATION OF INSECTS TO ITS TRANSMISSION

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INTRODUCTION

For the past 10 or 15 years the greatest annual loss to the truck growers in eastern Virginia has been due to the trouble known as "spinach-blight." This was supposed to be a malnutrition disease caused by improper fertilization, and previous recommendations for its control comprehended the improvement of soil, fertilizer, and cultural conditions.

The shipments of spinach (*Spinacia oleracea*) from the eastern Virginia trucking region have averaged between 600,000 and 1,000,000 barrels annually, valued at $1,000,000 to $1,500,000. Owing to the ravages of the blight during the past two years (1916–17) the acreage has been materially decreased, and many growers have abandoned the crop entirely, while others grow it only on newly cleared land. Spinach is second in importance as a truck crop in this region, being surpassed in acreage and value only by the potato (*Solanum tuberosum*). Spinach is grown during the winter, and thus utilizes land which would otherwise be idle at that season.

The conditions which prevail and the losses sustained from spinach-blight have been carefully estimated for several years. From the data thus collected it appears that blight annually destroys or renders unfit for use not less than 20 per cent of the spinach crop. A conservative estimate of the annual money loss to the eastern Virginia growers by this disease is between $200,000 and $400,000.

The writers have found that this disease occurs in the spinach-growing regions of New York and Ohio under the name "spinach-yellows." Increasing losses may be expected in those States as the disease becomes

[1] The name "spinach-blight" is used throughout this paper because this is the name by which this disease has been known since its first appearance in Virginia.

[2] Collaborator, Office of Cotton, Truck, and Forage Crop Disease Investigations, United States Department of Agriculture.

[3] Detailed by the Virginia Crop Pest Commission for the investigation of insects affecting truck crops.

more prevalent. It is not unlikely that spinach-blight is present in other States where this crop is grown either for truck or seed purposes.

The majority of the experiments recorded in this paper have been performed during the past three years, and the direct inoculation experiments, under the controlled conditions of field and greenhouse cages, have been conducted since the fall of 1916. As the problem is far from complete and many important points are as yet unsettled this paper has been prepared as a preliminary report dealing with the nature of the disease, its dissemination, and transmission by insects.

In this cooperative undertaking certain of the generalized points herein presented have been proved by each worker independently, and the results then compared. The experiments relative to the insect transmission of the disease, together with the determination of the conditions by which this is influenced and controlled, were performed by the entomologist. The experiments on the relationship of soil, fertilizer, and seed conditions to spinach-blight, and the various virus inoculations to determine the points relative to the nature of the disease were performed by the plant pathologist.

DESCRIPTION OF SPINACH-BLIGHT

Spinach-blight is a specific disease characterized by mottling and malformation of the leaves (Pl. A). Although having many of the symptoms of the mosaic diseases of tobacco, cucumber, etc., spinach-blight differs from them in that the affected plants are eventually killed. (Pl. 1, A.) The disease appears either on plants scattered over the field (Pl. 1, B) or on many adjacent plants, thus forming a distinct area.

This blight may be distinguished from the various fungus diseases with which it may be associated, such as downy-mildew, caused by *Peronospora effusa* (Grev.) Rbh. (Pl. 2, A), Heterosporium leafspot, caused by *Heterosporium variabile* Cke. (Pl. 2, B), and anthracnose, caused by *Colletotrichum spinaciae* Ell. and Halst. (Pl. 3, A), because no microscopic organism is found associated with it. Spinach-blight may also be distinguished from diseases produced by the above-named fungi, by its causing a gradual degeneration of the tissues instead of definite leafspots. It has been observed that blighted plants are more susceptible to the attacks of certain fungi than are adjoining healthy plants. That the blighted plants are lower in vitality is shown by the fact that such plants are most seriously injured by occasional cold periods during the winter (Pl. 3, B).

Owing to the appearances of the blighted plants at various periods in the course of the disease, it is difficult to give a description which would be inclusive of their appearance at all times. For convenience eight purely arbitrary stages have been selected, as they offer rather marked

changes in appearance. The following is a description of the appearance of the plant at each stage of the disease:

STAGE 1.—Plants in this stage may be distinguished from the healthy plants by a very slight yellowing which generally occurs on the younger leaves or those which are not fully opened. Occasionally a slight yellowing may be noticed on one or more of the older leaves; otherwise the plant is in a vigorous growing condition and still retains a normal dark coloration (Pl. 4, A).

STAGE 2.—Plants in the second stage of the disease have a more pronounced yellowing on the younger leaves than those in stage 1. The plants are usually vigorous, and no marked changes are to be noted in the older leaves. Occasionally the younger leaves begin to show evidence of being malformed. This, however, is not usual in this stage.

STAGE 3.—This stage is characterized by the appearance of malformation in the younger leaves. They become much wrinkled and narrowed and show decided mottling. The yellowing has usually spread to many of the older leaves. Plants in the third stage of blight are generally not as large as healthy plants of the same age.

STAGE 4.—Plants in this stage show a distinct evidence of stunting; the yellow color has spread over the entire plant, and the older leaves are distinctly mottled. The younger leaves, while still growing to a certain extent, are so malformed as to be hardly recognizable as spinach leaves. They are very finely savoyed and have a feathery appearance. They do not curl to any great extent. The plant has lost most of its vigor by this time, and little growth takes place after this stage.

STAGE 5.—The fifth stage is characterized by the disintegration of tissues, usually shown by a browning and dead appearance of parts of the older leaves; the browning usually occurs on the outer tips first and works inward as the disease progresses. The younger leaves may become wholly yellow. This color is lighter in the fall and winter than it is in the spring, when it occasionally shows as deep orange-yellow. The mottled appearance of the older leaves at this stage is very striking; such chlorophyll as remains is gathered along the veins, leaving the tissues between the veins a pale-yellow color. The older leaves become wrinkled and lose much of the deep savoying characteristic of healthy leaves.

STAGE 6.—The sixth stage (Pl. 4, B) is characterized by the total disintegration of the older leaves; they become brown and lose turgidity, often being supported only at the point of attachment, the remaining portions resting almost entirely on the ground. About this time the central leaves of the plant begin to turn brown. The older leaves pass from a brownish yellow to a more or less translucent brown or straw color.

STAGE 7.—The older leaves are practically disintegrated, with nothing but the petioles remaining. The younger leaves are brown, with no evidence of green and very little yellow color. The plant at this stage has reached a point of low vitality, but life still continues in the younger leaves and crowns. This is shown in Plate 5, B, *a*.

STAGE 8.—The plant is dead, but has not entirely disintegrated (Pl. 5, B, *b*). At this stage a plant which was perhaps 15 inches in diameter before it was attacked by the disease is reduced to a small whorl of leaves scarcely an inch in diameter.

The root is apparently healthy and performs its normal function until the fourth stage is reached. From the fourth to the eighth stage the root gradually declines from the normal appearance. The root of a diseased plant is characterized by its shrunken appearance, a loss of lateral rootlets, and a browning of the internal tissues. This would indicate that the disease affects the foliage and aerial portions of the plant rather than the roots, the effect on the roots in all probability being secondary.

THE LENGTH OF THE VARIOUS STAGES

A more or less definite length of time elapses between the appearance of the first symptoms of the disease and the death of the host. In order to determine the length of the life cycle of the diseased plants under field conditions, the following experiment was performed.

Forty-one healthy and diseased plants were staked in the field on February 9, 1917. Records were made of the condition of the plants and the stage of progress of the disease. They were examined on February 22, March 3 and 22, April 2, 10, and 25. Each time records were made of the condition of each individual plant. The results of these observations are shown graphically in figure 1. The incubation period, or the time elapsing between inoculation and the appearance of the first symptoms under field conditions, was determined from the inoculation experiments. The average time between the inoculation and the death of a spinach plant is 81.75 days, and the disease progresses gradually from the appearance of the first symptoms until the plant is dead. The slight variation shown in the length of the different stages is probably due to the variations in the time the observations were made. Theoretically the line representing the progress of the disease would be straight.

HISTORY OF SPINACH-BLIGHT

Spinach-blight was first observed at Lambert's Point, Norfolk County, Va., about 13 years ago and has since spread throughout the entire section.

Prior to 1907 spinach-blight had become so serious that spinach growers were asking for assistance in its control. The first experiments that were conducted with regard to the control of spinach diseases in Tidewater, Va., were undertaken by the Office of Cotton and Truck Dis-

ease Investigations of the United States Department of Agriculture. The first published reports on these experiments were issued as Bulletin No. 1 of the Virginia Truck Experiment Station in September, 1909.[1] From a discussion of the symptoms of the diseases studied at that time it would appear that the true spinach-blight was included. However, there were undoubtedly other diseases of truck crops which resembled spinach-blight in appearance, and the results obtained since that time would indicate that no distinction was made between them, all being classed as malnutrition diseases.

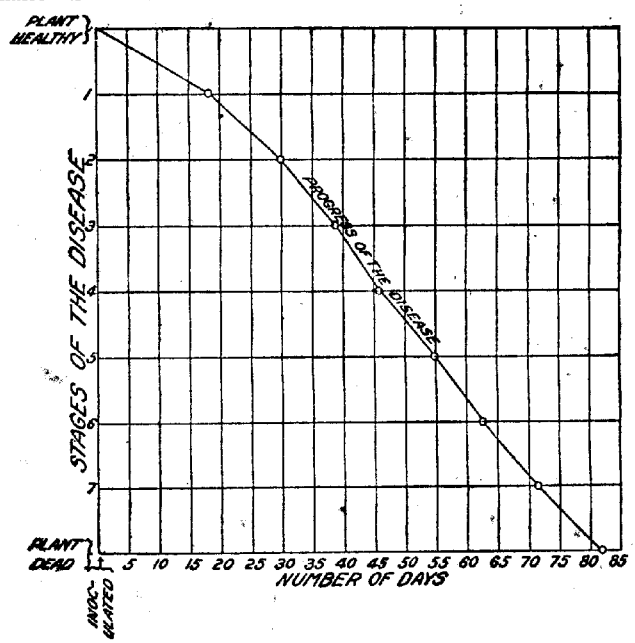

FIG. 1.—Graph representing the average lengths of the various stages in the progress of spinach-blight.

The work which was begun by Mr. L. L. Harter, of the Bureau of Plant Industry, was continued and published as Bulletin 4 of the Virginia Truck Experiment Station.[2] While this bulletin gave valuable suggestions for spinach culture, nevertheless it did not solve the spinach-blight problem. These data furnished the basis for distinguishing malnutrition diseases, which could be corrected by the use of proper fertilizers and lime, from the true spinach-blight, which subsequent experiments have shown has little or no relation to the fertilizer and lime content of the soil.

[1] HARTER, L. L. THE CONTROL OF MALNUTRITION DISEASES OF TRUCK CROPS. Va. Truck Exp. Sta. Bul. 1, 16 p., 4 fig. 1909.

[2] ——— SPINACH TROUBLES AT NORFOLK AND IMPROVEMENT OF TRUCKING SOILS. Va. Truck Exp. Sta. Bul. 4, p. 61–80, fig. 18–22. 1910.

CIRCUMSTANTIAL EVIDENCE OF THE NATURE OF THE DISEASE

For some years past growers have observed that spinach-blight followed within a reasonably short time after serious outbreaks of aphids. These observations pointed toward the possibility that aphids were the direct cause of blight. Recent experiments disprove this theory, and at the same time explain the indirect relation between the outbreaks of aphids and the subsequent epidemics of spinach-blight.

INOCULATION OF FIELD PLANTS WITH THE JUICE FROM BLIGHTED PLANTS IN THE WINTER OF 1915–16

In Bulletin 4 of the Virginia Truck Experiment Station,[1] Harter called attention to the fact that spinach-blight resembled the mosaic disease of tobacco, and plants in certain stages of spinach-blight have decidedly mottled leaves, quite characteristic of mosaic diseases. These observations, together with the indication that no microscopic organism appeared to be the cause of spinach-blight, led to the inoculation of healthy field plants with the juice of blighted plants. A bed of plants about one-half the size of marketable spinach was selected for this experiment. A quantity of blighted plants were collected on an adjoining farm, and the juice was obtained by mashing the tissues and straining through cloth. To serve as controls two rows of plants in the above-mentioned bed were pricked with a flamed needle, care being taken to wound both young and old leaves. The other rows of plants were inoculated by placing drops of juice from the diseased plants on leaves of various ages and pricking the juice into them with a flamed needle. These were kept under observation until they were large enough to harvest. No attempt was made to cover any of these plants, or to keep insects from them; but up to the time of inoculation no signs of blight had appeared in this bed or in any of the adjoining beds. Not long after the inoculations had been made, an outbreak of aphids occurred throughout the entire section. The plants on many farms became so badly infested that it injured their value for market purposes. Data collected from time to time showed that spinach-blight had developed on the inoculated plants. Blight also occurred to a limited extent on the control plants. This experiment gave merely an indication that spinach-blight is a communicable disease, and is spread in some manner, possibly by aphids, possibly by needle pricks when the juice is thus artificially carried, or possibly by some unknown agent.

RELATION OF DRAINAGE TO SPINACH-BLIGHT

It was the opinion of some growers that spinach-blight was directly associated with poor soil drainage; therefore the assistance of Dr. J. A. Bonsteel, of the Bureau of Soils of the United States Department of Agriculture, was obtained, and the relation of this factor to spinach-

[1] HARTER, L. L. *op. cit.*, 1915.

blight determined as follows: Typical blighted areas in several fields were selected, and soil borings made to determine the texture and condition of both surface and subsoil. Similar borings were made in the same fields in areas where the plants appeared healthy. In no case was it found that the drainage was poor where the plants were blighted, and in some cases the subsoil in these areas was such that the drainage was much better within the blighted areas than in parts of the field where the plants appeared healthy. This would indicate that the matter of drainage has little or no direct bearing on the cause or spread of spinach-blight.

FERTILIZER EXPERIMENTS

RELATION OF FERTILIZERS TO THE CONTROL OF SPINACH-BLIGHT IN 1915

At the suggestion of Mr. L. L. Harter experiments were begun in which stable manure at the rates of 20 and 30 tons per acre and lime and gypsum at the rate of 1 ton to the acre were used in an attempt to determine the relation of these fertilizers to spinach-blight. Plots 0.1 acre in size were treated with the different substances, and similar untreated plots served as controls. The plots were planted with seed from the same lot and given similar cultural conditions. Spinach-blight developed as abundantly on the various treated plots as on the control plots; therefore it would appear that stable manure, lime, and gypsum have no influence on controlling spinach-blight.

SOIL AND FERTILIZER EXPERIMENTS ON SPINACH-BLIGHT CONTROL[1]

The relation of soils and fertilizers to spinach-blight was unsettled previous to 1915; therefore cooperative experiments were begun. A series of plot experiments were outlined which was to include various individual fertilizer elements and also combinations of fertilizers and complete fertilizers made up on acid and basic principles. Forty plots were used in this experiment. Blight was equally abundant on all of the plots. No data were obtained to indicate that any of the individual elements or combinations of elements had any direct relation to the control of spinach-blight.

Certain modifications of the above experiments were repeated in 1916. These substantiated the 1915 results, that fertilizers have no direct relation to spinach-blight.

RELATION OF FUNGI AND BACTERIA TO SPINACH-BLIGHT

Although previous workers had obtained results which indicated there was no specific fungus or bacterium which was the cause of spinach-blight, nevertheless it seemed advisable to repeat this work. Blighted spinach plants were collected from various fields and from different areas in the

[1] Conducted in cooperation with the Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture.

same field. Sections of diseased tissues from various parts of blighted plants were externally disinfected, and then plated on various types of nutrient media. The plates were incubated at various temperatures until organisms began to develop on the plates. Each type of organism was then transferred to agar slants, and grown in pure culture until its characteristics had developed. Both bacteria and fungi were isolated from various tissues of blighted plants. Plants in more advanced stages of blight yielded an abundance of organisms, while tissues from some of the plants in early stages of the disease remained sterile on the plates, thus indicating that no microscopic organisms were present in the tissues. Examination of the various cultures showed that some common organisms including species of Alternaria, Fusarium, Verticilium, Rhizoctonia, and Macrosporium were present, together with various of the lower soil fungi. Bacteria of numerous types and colors were also isolated from diseased tissues, but none of these appeared different from the common-soil and decayed-plant-infesting organisms. Subcultures of these organisms were prepared for inoculation work in the field, and with these a number of healthy field plants were inoculated by pricking the organism into the healthy tissues. A sufficient number of plants were used as controls. The inoculated plants and controls were under observation for a long time, but in no case did any of the inoculated plants consistently show a higher percentage of blight than the controls. These results led to the conclusion that none of the organisms which had been isolated in the laboratory from blighted plants was the specific cause of spinach-blight. The fact that the tissues from certain of the blighted plants, when externally disinfected and plated, remained sterile indicated that the disease could occur in a plant without any microscopic organism being associated with it. Therefore these results confirmed those of previous workers to the effect that spinach-blight is a definite disease, and is not due to any specific microscopic organism thus far obtained.

METHODS EMPLOYED IN THE SPINACH-BLIGHT EXPERIMENTS IN 1916–17

The cages used in the first experiments were furnished by the United States Department of Agriculture, but they were found to be too large for convenient work. The door was small, and the operator did not have room to reach conveniently to the corners of the cage. Therefore the cages used later[1] were smaller, the dimensions being 2½ by 3 by 2 feet; the frame was made of 1-inch material and the door occupied one whole side. This made an arrangement whereby the operator had access to all parts of the cage; yet the cloth was draped around him sufficiently to eliminate the possibility of the insects' gaining entrance through the large opening (Pl. 5, A). The door and manner in which it is made is shown in Plate

[1] The writers wish to acknowledge the suggestions of Mr. J. B. Norton, of the Office of Cotton, Truck and Forage-Crop Disease Investigations, Bureau of Plant Industry, in the development of the same.

6, A. The cloth used to cover the cages is known as light-weight sheeting. For aphid experiments it is necessary to use cloth with a close weave and yet not so close as to prevent a free circulation of air.

A type of small cage was developed which could be used to cover individual plants, or pots of plants on which insects were placed within the larger cages. These are shown in Plate 6, B. They consist of 12-mesh wire screen, rolled to a cylinder, and covered with a fine grade of sheeting or surgeon's gauze. Those with a diameter of 4, 6, or 10 inches were found most convenient. For use with spinach a convenient height is 12 inches, although for taller growing plants, as peppers and tomatoes, a height of 24 inches is preferable. The cloth is allowed to extend 7 or 8 inches beyond the top of the cylinder. It may then be drawn toward the center, gathered, and tied with a string, which permits the cylinder to be set over a pot and the plants examined through the opening made by loosening the string at the top. The cylinders were forced about 1 inch into the soil to prevent insects entering from below.

All individual plants or pots of plants within the larger field cages, on which aphids were placed, were covered with the small individual cages. At the end of the time the aphids were allowed to remain on experimental plants, the cages were fumigated with nicotine to kill the aphids. The plants were then carefully examined to insure that all the aphids had been killed and removed. Control plants were grown in each field cage, in order that the controls on each series might be under similar conditions of temperature, humidity, and soil. Records of conditions of temperature and humidity were made both within and without the field cages. The humidity was determined by means of wet and dry-bulb thermometers kept about 3 inches above the surface of the soil.

As no specific microscopic organism had been found associated with spinach-blight, the only means of proving infections was by further inoculations from the artificially infected plant, either by aphid transfers or by needle pricks. As certain forms of chlorosis (Pl. 10, B) and malnutrition, produced either by the lack of fertilizers or by the excessive applications of them, may cause the spinach plants to become similar in appearance to those affected with blight, the appearance and color of the plant could not be taken as positive evidence of the presence of the infectious disease. Two to five healthy plants were inoculated with the virus. When one or more of these plants developed a mottled appearance of the leaves characteristic of the disease, the original plant was credited with positive symptoms at the time the first color changes became visible in its leaves. Most of the secondary inoculations were performed in the greenhouse. As the season advanced and larger series, with higher percentages of infection occurred, only 5 to 50 per cent of the plants in each series were checked out in this manner. In the majority of cases the prick inoculations were made in the true leaves, but

in a few instances where the plants used were very young it was necessary to make the inoculations in the cotyledon leaves.

It was noticed in making the transfers of aphids to young spinach plants that they generally fed on the under side of the leaves rather than on the stems or petioles; hence, it is probable that in the majority of cases the inoculations were made from that point, when virus-bearing aphids were transferred to healthy spinach plants.

In making transfers or in any way handling the experimental plants the greatest care was exercised to keep the hands and instruments disinfected. Ninety-five per cent alcohol was used for this purpose. Great care was also exercised when making transfers of insects from diseased to healthy plants. The insects were carefully removed from a diseased plant by means of a camel's-hair brush, and were then placed on sterile glass slides on which they were carried to the healthy plants. They were removed from the slide by jarring it slightly. In this way neither instrument nor hand came in contact with the healthy plant. Needles and other instruments used in making prick inoculations were carefully flamed between each operation. In the field cages certain soil-inhabiting insects would occasionally gain access to the spinach plants and oftentimes attack them. This was almost entirely eliminated by thoroughly drenching the soil in the cage with a 1 to 100 solution of 40 per cent nicotine sulphate at the time it was set in the field.

In the majority of cases the precaution was taken to disinfect the spinach seed in 1 to 100 formaldehyde for a few minutes prior to planting. The majority of plants used in the greenhouse were grown from seed planted in steamed soil.

INSECTS COMMONLY ASSOCIATED WITH SPINACH AT THE TIME BLIGHT IS PREVALENT

Two species of Aphididae are primarily associated with spinach at the time blight is prevalent. These are the potato aphis (*Macrosiphum solanifolii* Ashmead) and the spinach aphis (*Rhopalosiphum persicae* Sulzer). Blight occurs most abundantly between the 1st of November and the 1st of April, and these aphids are prevalent at all times during the winter months. As will be shown, several other species of insects are capable of transmitting spinach-blight from diseased to healthy plants, but they are of minor importance in this connection. Thus far, these two species of aphids have been found to occur in Tidewater, Va., only as viviparous females. The true sexes have neither been collected nor reared. From our studies of the species it has been found that *M. solanifolii* is more active and reproduces more readily during periods of low temperature than *R. persicae*. Laboratory studies relative to these facts have been confirmed by field observations. The feeding habits of the two species are somewhat different. *M. solanifolii* are ungregarious, the alate and apterous forms move readily from plant to plant during

favorable weather, and may feed on many plants during their life. For this reason heavy infestations of this species within localized areas in a given field are rare. *R. persicae* has a tendency to congregate and, except during periodic outbreaks, are abundant only in more or less restricted areas within a field. As might be expected, the alate forms are the more active disseminators of spinach-blight.

Conditions which lead to a large production of alate females are indirectly responsible for the epidemics of blight which may follow. The production of alate females of *M. solanifolii* occurs throughout the winter months, and counts made at various times during the season showed that the ratio of alate to apterous forms was 4.12 to 1 for 2,500 individuals. Alate females of *R. persicae* are less numerous than the apterous form, except during a severe outbreak; under normal conditions the ratio of abundance of the two forms in the order mentioned is 1 to 47.41 for 2,500 individuals. Counts made at the time of an outbreak showed that the two forms were about equally abundant.

From its ability to withstand lower temperatures *Macrosiphum solanifolii* does not undergo the numerical fluctuations which occur with *Rhopalosiphum persicae*. The latter species occurs in great abundance, usually two or three times during the winter. Severe epidemics of spinach-blight often follow within a few weeks in the areas of heavy infestation.

FOOD PLANTS OF MACROSIPHUM SOLANIFOLII AND RHOPALOSIPHUM PERSICAE

Rhopalosiphum persicae attacks nearly all vegetable crops, as well as many weeds, shrubs, and trees. Over 60 species have been listed as its food plants, and undoubtedly there are others which are yet unknown.

Dr. Edith M. Patch,[1] in reporting her studies of *M. solanifolii*, gave a list of 20 plants which served as its food. In our studies of the species under southern conditions it has been found to be more cosmopolitan in its feeding habits than was hitherto supposed. The question of the relationship between the food plants of both *R. persicae* and *M. solanifolii* to spinach-blight has entailed a great amount of work which is as yet unfinished; hence, aside from mentioning that spinach is the most important fall and winter food plant of both species in this region, the findings in this connection are reserved for a later publication.

DIRECT INOCULATIONS WITH THE JUICE FROM BLIGHTED PLANTS

VIRUS INOCULATIONS

Blighted plants were collected from a field of young spinach and brought to the laboratory, where they were mashed in a mortar and the juice strained through a cloth. With a flamed needle the first row of

[1] PATCH, EDITH M. PINK AND GREEN APHID OF POTATO. Maine Agr. Exp. Sta. Bul. 242, p. 205–223, fig. 47–49. 1915.

plants in a field cage was pricked, and the juice from the blighted plants then sprayed on them with an atomizer. The wet leaves were again pricked with a flamed needle to insure the entrance of the virus into the tissues. An adjoining row of plants in the same cage was pricked with a flamed needle to serve as controls. Sixty-two days after inoculation blighted plants were observed in row 1, while the row of control plants appeared healthy.

Eighty-nine days after inoculation a mottled leaf was removed from a blighted plant in row 1, and six plants in one pot in the greenhouse were inoculated with it by mashing the blighted tissues into the healthy leaves of the seedlings. Several pots of plants similar to the above were pricked with a flamed needle to serve as controls. Thirty-one days after inoculation the six plants were in the advanced stages of blight, but the control plants remained healthy. These results indicate that the disease is due to a specific virus which is capable of producing the characteristic symptoms of blight both in the greenhouse and the field plants.

One typical blighted plant from a large area was selected for inoculation purposes. This plant was mashed, and the juice strained through a cloth and used to inoculate one large potted plant in the greenhouse by needle pricks. A similar plant in another pot was pricked with a flamed needle and served as a control. Thirty days later the inoculated plant had developed typical symptoms of blight, while the control plant remained healthy. Nine days later individuals of *R. persicae*, which were known to be free from infection were placed on the diseased plant. Twenty-five days later aphids were removed from the above plant and placed on leaves of three large spinach plants growing in a field cage. Similar plants in the same field cage were used as controls. Twenty-one days later the control plants appeared healthy, but the three inoculated plants had developed typical symptoms of blight. These results indicate that the plants in the large area had typical spinach-blight, and that the virus from these plants produced the disease in greenhouse plants; further, that when aphids free from infection were allowed to feed on blighted greenhouse plants they were able to transfer the infectious entity to plants in field cages.

INOCULATIONS WITH A WATER SUSPENSION OF VIRUS FROM BLIGHTED PLANTS

A water suspension of blighted plants was made by mashing the mottled leaves in tap water. This suspension was strained through a cloth into an atomizer. With a flamed needle a row of plants in a field cage were pricked, and then sprayed with the contents of the atomizer. The wet leaves were again pricked with a flamed needle to insure the virus entering the tissues. Another row of plants in the same cage was pricked with a flamed needle and served as a control. Twenty days

after inoculation the first blighted plants were observed. All of the control plants appeared healthy. Twenty-seven days after observing the first blighted plants a mottled leaf was removed from one of them, and with it seedling spinach plants in a pot in the greenhouse were inoculated by mashing the diseased tissues into their leaves. A similar pot of seedlings was pricked with a flamed needle and served as a control. Thirty-one days later three of the seedling plants had developed decidedly mottled leaves, typical of blight. All of the control plants appeared healthy. These results indicate that the original blighted plants contained a virus which, when extracted in water and inoculated into other field plants in the outdoor cages, produced a condition similar to the original blighted plants. Leaves from these artificially infected plants contained a virus which produced typical symptoms of blight in plants growing in the greenhouse, thus indicating that it is not unfavorable soil nor temperature conditions which cause the disease in the field or in the greenhouse.

DIRECT INOCULATIONS WITH THE JUICE OF BLIGHTED PLANTS IN WHICH THE DISEASE HAD BEEN PRODUCED BY APHIDS

Blighted plants were collected from two large areas. Alate and apterous *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were removed and placed on spinach seedlings in a field cage. Sixty-one days after transferring the aphids typical symptoms of blight appeared on two of the plants. Thirteen days later one more plant developed mottled leaves typical of blight. On the same day one mottled leaf was removed from each of the two blighted plants first observed in this cage, and was brought to the greenhouse for inoculation purposes. Ten spinach plants 49 days old were inoculated as follows: With a flamed needle, the juice from the mottled leaf of plant 1 was pricked into the healthy leaves of five seedlings in two pots, the other five seedlings in two pots being inoculated by mashing the blighted tissues into the leaves. Ten other spinach plants in four pots were inoculated in the same manner with a flamed needle and the mottled leaf from blighted plant 2. Two similar spinach plants in separate pots were pricked with a flamed needle to serve as controls.

Twelve days after inoculation, one of the potted plants had developed the mottled leaves characteristic of blight; the other inoculated plants and the controls appeared healthy. Sixteen days after inoculation a photograph was taken to show the difference in appearance between the inoculated plants and a control (Pl. 7, B). Twenty-four days after inoculation 13 of the 20 inoculated plants had developed mottled leaves characteristic of blight. The remaining inoculated plants, although not showing the mottled leaves, did not have the healthy appearance of the control plants.

These results indicate that the aphids which had fed on blighted plants carried the virus to healthy field plants. That the aphids were only carriers of the virus and not the true cause of the disease is shown by the fact that the virus from the plants artificially infected by the aphids produced typical symptoms of the blight when transferred by needle pricks to healthy plants in the greenhouse.

DIRECT VIRUS INOCULATIONS FROM FIELD TO GREENHOUSE AND BACK TO FIELD

Aphids were removed from blighted plants and placed on three pots of spinach seedlings. Subsequently the blighted plants were mashed in a mortar and the juice pressed out through a cloth. Some of this juice was pricked into seedling plants which were then placed under a globe in the greenhouse to keep them under more humid conditions. Twenty-one days after inoculation two of the plants had developed characteristic symptoms of the blight. A similar pot of plants, pricked with a flamed needle, covered with another globe, and used as a control, remained healthy. With some of the extracted juice, three plants in separate pots were inoculated by pricking the virus into the leaves. A similar pot of plants was pricked with a flamed needle to serve as a control. Eleven days after inoculation symptoms of spinach blight began to develop in three plants. Twenty-one days after inoculation, two of the plants were in advanced stages of blight, while the third was considerably stunted, but not as mottled as the two others. Eighty-six days after inoculation four large plants in a field cage were inoculated by mashing into them a leaf from one of the above blighted plants. Similar plants in the same cage were pricked with a flamed needle and served as controls. Eighteen days after inoculation three of the four plants had developed typical symptoms of blight, while the control plants appeared healthy.

DIRECT INOCULATIONS BY APHIDS PROVED BY VIRUS INOCULATIONS

Individuals of *Rhopalosiphum persicae* and *Macrosiphum solanifolii* were shaken from blighted plants and placed in a large pot containing over 100 spinach seedlings. Twelve days later distinct mottling of some of the spinach leaves was observed. Sixteen days after inoculation numerous plants had decidedly mottled leaves. Ten days later a large percentage of the 100 plants in this pot had developed typical symptoms of blight.

Twenty-[illegible] days after inoculation one leaf was removed from each of three blighted plants in the above pot, and with these mottled leaves seedlings in two pots were inoculated by pricking and mashing the blighted tissues into them. A third pot of six plants was inoculated by pricking juice from one of the blighted leaves into the seedlings. A

similar pot of seedlings was pricked with a flamed needle and served as a control. Seven days after inoculation the two pots, of two and four plants, respectively, developed typical symptoms of blight. Nine days after inoculation the six plants in one pot developed doubtful symptoms and five days later all of these plants had developed positive symptoms of blight. Twenty-one days after inoculation all of the above plants were in advanced stages of blight, while the control plants remained healthy. These results indicate that the aphids from the original blighted field plants served as carriers of the virus, because the plants which blighted as a result of the feeding of the aphids on them contained a virus which produced the disease when pricked into healthy spinach plants.

INOCULATIONS WITH THE TISSUES OF BLIGHTED PLANTS IN WHICH THE DISEASE WAS PRODUCED BY APHIDS

A number of *Rhopalosiphum persicae* and *Macrosiphum solanifolii* were removed from blighted plants and placed on a pot of seedling spinach 13 days old. This pot contained more than 100 seedlings. A similar pot of seedlings served as a control. Eighteen days after inoculation a few plants in the above pot had developed typical symptoms of blight. Thirty-four days after inoculation the majority of the inoculated plants had developed symptoms of the disease, while the control plants still appeared healthy. The majority of the inoculated plants eventually became much stunted and many died, but the control plants remained healthy (Pl. 8, A).

Eighteen days after inoculation two of the blighted plants were removed from the above pot and two seedling spinach plants were inoculated with them by pricking through the mottled leaves into the leaves of the seedlings with a flamed needle. A third plant in another pot was pricked with a flamed needle to serve as a control. These pots of plants were placed in a cage in the greenhouse. With the two blighted plants used for the greenhouse inoculations six plants in a field cage were inoculated by pricking through the blighted leaves into the leaves of the healthy plants. Seventy-nine days after inoculation four of the six caged field plants showed positive symptoms of spinach blight. Fourteen days after inoculation the two seedlings in the pot in the greenhouse had developed mottled leaves, while the control remained healthy. Eighty-four days after inoculation a photograph was taken to show the appearance of the control as compared with the inoculated plants (Pl. 8, B). These results indicate that spinach-blight is due to a specific virus which is readily transmitted from the field to the greenhouse, and vice versa, either by means of aphids which have fed on diseased plants or by transferring the juice of the diseased plants by needle pricks.

EARLY FIELD EXPERIMENTS[1] ON THE INSECT TRANSMISSION OF SPINACH-BLIGHT, 1916–17

The following data were collected from experiments performed in December, 1916, and January, 1917. The plants used in the experiments had been growing in large field cages for about three weeks previous to the time the inoculations were made. Large-sized lantern globes (Pl. 10, A) were used to cover the plants during the 48-hour period which the aphids were allowed to remain on them. Adult apterous females were used in the transfers of aphids from the diseased to healthy plants.

DIRECT TRANSFERS OF APHIDS FROM DISEASED TO HEALTHY PLANTS

SERIES 1

INOCULATIONS WITH MACROSIPHUM SOLANIFOLII.—Three plants in a field cage were selected and on the first were placed 10, on the second 10, and on the third 20 individuals of *M. solanifolii* which had previously been feeding on diseased spinach. Positive symptoms of blight developed on all three plants in 22, 17, and 22 days, respectively. The mean temperature inside the cages for the 22 days of the incubation period was 45° F. Outside the cages the mean temperature was 42°.

INOCULATIONS WITH RHOPALOSIPHUM PERSICAE.—Another series of three healthy plants were inoculated by placing on them 10, 20, and 10 individuals of *R. persicae*, respectively, from diseased plants. The typical mottling and malformations due to the disease appeared on all three of the plants 22, 29, and 22 days after the transfers were made. This experiment was conducted in the same cage as the first. No disease appeared on four untreated plants used as controls.

SERIES 2

Series 2 was started in January, 1917, and the results are shown in Table I. On January 17 thirty adults of *Macrosiphum solanifolii* which had been feeding on lettuce were placed on plant 1 of series 1, and 30 adults of *Rhopalosiphum persicae* were placed on plant 4 of the same series. The aphids were allowed to feed for three days on these plants before the transfers were made. Individuals of *M. solanifolii* from diseased plant 1 were placed on three healthy plants. After feeding for 48 hours the aphids were removed. Positive symptoms of the disease developed in three of the plants 16, 16, and 23 days after inoculation. Three plants were inoculated by needle pricks with virus taken from plant 1. All three plants gave positive symptoms of the disease on the sixteenth day. Ten adults of *M. solanifolii* born and reared on lettuce (*Lactuca sativa*) were placed on each of three healthy spinach

[1] During the summer of 1916 cooperative experiments were planned between the Entomologist of the Virginia Truck Experiment Station and Mr. D. E. Fink, of the Office of Truck Crop Insect Investigations of the Bureau of Entomology. This work was started in the fall of 1916 on a spinach field on the Station farm. Successful transmissions of blight from diseased to healthy plants were obtained, and some data relative to the character of spinach blight collected.

seedlings. After remaining on the plants for 48 hours, the aphids were removed. No cases of blight had developed at the time the experiment closed, 54 days after the transfers were made, thus indicating that the aphids from lettuce did not carry infection. Ten control plants which were untreated remained healthy. Ten adults of *R. persicae* taken from plant 4 were placed on three healthy spinach plants. All three developed blight on the sixteenth day. Three plants were inoculated by needle pricks with the virus from plant 4. Two of the plants developed positive symptoms of the disease on the fifteenth day. The third remained healthy until the experiment closed. Forty adult *R. persicae*, the offspring of individuals which had been feeding on lettuce for three generations, were distributed equally on four healthy spinach plants. They remained on these plants for a period of 48 hours and were then removed. One plant developed typical symptoms of blight 23 days after it was inoculated. The three remaining plants were still healthy when the experiment closed. Ten plants which were untreated and used as controls remained healthy.

TABLE I.—*Results of field experiments on the insect transmission of spinach-blight (series 2), Norfolk, Va., 1917*

Plant No.	Source of diseased material.	Method of transmission.	Species of insect used.	Incubation period.	Mean temperature in cages during incubation period.
				Days.	°*F*
1	Plant 1, first series 1916 experiments.	10 live aphids	*Macrosiphum solanifolii.*	16	46
2	do	8 live aphids	do	16	45
3	do	12 live aphids	do	23	43.2
4	do	Needle pricks with diseased tissue.		16	45
5	do	do		16	45
6	do	do		16	45
7	Controls untreated			Healthy.	
8	do			do	
9	do			do	
10	Aphids reared for two generations on lettuce.	10 live aphids	*M. solanifolii.*	do	
11	do	do	do	Healthy.	
12	do	do	do	do	
14	Plant 4, first series	do	*Rhopalosiphum persicae.*	16	45
15	do	do	do	16	45
16	do	5 live aphids	do	16	45
17	do	Needle pricks		15	45
18	do	do		15	45
19	do	do		Healthy.	
20	Controls untreated			do	
21	do			do	
22	do			do	
23	Aphids, third generation, on lettuce.	10 live aphids	*R. persicae.*	23	43.2
24	do	do	do	Healthy.	
25	do	do	do	do	
26	do	do	do	do	

The results obtained in these early experiments indicated that the pathogenic agent of spinach-blight is extremely virulent and can easily be transmitted from one plant to another by aphids which pass from a diseased to a healthy plant. It is also transmissible to healthy plants by means of needle-prick inoculations. The incubation period, or the time elapsing from the inoculation to the appearance of color changes in the host, varied from 16 to 23 days, under the conditions present. One case of blight appeared on a plant to which lettuce-fed aphids had been transferred. Similar infections occurred about the same time in another set of field experiments being conducted in other cages.

The following field experiments were begun in 1916. Holland-grown seed was sown in December in field cages. The seedlings had developed the second pair of true leaves at the time the inoculations were made. The diseased material used for inoculation purposes was collected from a typical diseased area, located on a farm about ¼ mile distant from the Station property. Adult apterous individuals of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were transferred to the healthy plants. The inoculation period or the length of time the aphids remained on the healthy plants was 48 hours in each case. The methods employed in transferring the aphids and in making the inoculations are discussed elsewhere. The inoculations in the following experiments were made on January 10, 1916, and the results are given in Table II.

TABLE II.—*Results of field experiments on the insect transmission of spinach-blight at Norfolk, Va., 1916–17*

Method of inoculation.	Species used.	Number of insects per plant.	Number of plants used.	Number of plants infected.	Percentage of infection.	Average period of incubation.
						Days.
Live virus-bearing aphids	*Macrosiphum solanifolii.*	10	12	11	91.6	17.8
Do	*Rhopalosiphum persicae.*	10	12	11	91.6	17
Juice of virus-bearing aphids pricked into healthy plants.	*M. solanifolii*	.....	12	6	50	21.1
Do	*R. persicae*	.....	12	3	25	24.6
Virus pricked into healthy plants.	.....	.....	12	9	75	20.6
Pricked with sterile needle	Controls	.....	12	0	0	0
Nonvirus-bearing aphids born and reared on lettuce.	*M. solanifolii*	10	10	2	20	25.5
Nonvirus-bearing aphids born and reared on spinach.	*R. persicae*	10	10	3	30	27
Control plants	Uninoculated	.....	84	0	0	0

DIRECT TRANSFERS OF MACROSIPHUM SOLANIFOLII FROM DISEASED TO HEALTHY PLANTS

On each of 12 vigorous plants growing in field cages were placed 10 adults of *Macrosiphum solanifolii* from diseased plants. Eleven of the twelve plants developed positive symptoms of the disease. The incubation period varied from 12 to 30 days, the average being 17.8 days. One plant remained healthy until March 2, when the last record was made.

DIRECT TRANSFERS OF RHOPALOSIPHUM PERSICAE FROM DISEASED TO HEALTHY PLANTS

Another series consisted of 12 plants inoculated in a similar manner, except that 10 adults of *Rhopalosiphum persicae* were placed on each of 12 healthy plants. The aphids were collected on the same diseased plants from which a number of *Macrosiphum solanifolii* were taken for use in the first series. Again eleven plants developed symptoms of the disease between 12 and 30 days after they had been inoculated. The average incubation period was 17 days, or 0.8 day less than for the *M. solanifolii* series.

INOCULATIONS WITH THE JUICE OF CRUSHED APHIDS COLLECTED FROM DISEASED SPINACH

Twelve healthy plants were inoculated with the juice of crushed *Macrosiphum solanifolii* collected on diseased plants. The inoculations were made by means of needle pricks. Six plants became infected in an average period of 21.1 days. Likewise 12 plants were inoculated with the juice of crushed *Rhopalosiphum persicae* from diseased plants. These innoculations resulted in three infections in an average time of 24.6 days.

INOCULATIONS WITH VIRUS FROM PLANTS UPON WHICH THE APHIDS FED

Another series f 12 plants were inoculated with the virus taken from the crushed leaves of diseased plants upon which the aphids were feeding before they were transferred to healthy plants. The virus was pricked into the leaves by means of a sterile needle. Nine plants developed symptoms of blight in an average time of 20.6 days. As a check on this series 12 plants were pricked with a sterile needle. No cases of infection resulted, and the plants remained healthy until the experiment was closed.

TRANSFERS OF APHIDS BORN AND REARED ON LETTUCE TO HEALTHY SPINACH

As a control on the previous transfers made with insects from diseased spinach, 10 healthy plants were selected, and on them were placed a number of adults of *Macrosiphum solanifolii* born and reared on lettuce, and which had not fed on spinach until transferred to the plants in this series. The parents of these aphids were collected in the field from supposedly healthy spinach. Two of the 10 plants developed positive symptoms of blight, the first appearing on the twenty-first and the second on the thirtieth day after the transfers had been made. The eight

remaining plants were healthy on March 2. A duplication of this experiment was made with *Rhopalosiphum persicae* which had been born and reared on supposedly healthy spinach in the greenhouse. The parents of these aphids were collected from a field of apparently healthy spinach which at the time gave no evidence of the presence of the blight. The aphids were placed on spinach in the greenhouse and allowed to remain on the seedlings until there was a sufficient number of adults to use in this experiment. Four days after the transfers had been made to the outdoor cages, or 29 days after the original aphids had been brought to the greenhouse, six of the seedlings on which they had been placed became diseased. In the outdoor series three plants were infected, the symptoms developing in an average time of 27 days.

About 10 plants in each of the field cages remained untreated and were used as controls. There were 84 plants used in this manner, and all remained healthy until the experiment closed on March 2.

The results obtained through the transfers of live aphids from diseased to healthy plants proved the ability of the aphids to transmit the disease from plant to plant in the field. The ability of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* to transmit the disease was about the same, although the incubation period for plants inoculated by *M. solanifolii* was 0.8 day longer than the average incubation period of plants inoculated by *R. persicae*. The juice obtained from crushed aphids from blighted plants proved to be infectious; that obtained from *M. solanifolii* gave 50 per cent and that from *R. persicae* 25 per cent of positive infections. It is noticeable that in this case the average incubation period was one to seven days longer than where the inoculations were made through either the feeding punctures of the aphids or by pricking the expressed virus into the plant tissues. The peculiar results which were obtained by the transfers of lettuce-fed aphids to healthy plants are not easily explained. It was known that the *M. solanifolii* had not come in contact with diseased spinach from the time of their birth until they were transferred to the healthy spinach plants, and yet they produced positive cases of blight in 2 cases out of 10 inoculations. *R. persicae* from supposedly healthy spinach plants produced 3 cases of blight out of the 10 plants inoculated. It will be noticed that the average incubation period of the disease in all five of these positive cases was 8 to 10 days longer than the average incubation period of the disease when caused by the direct transfers of aphids from diseased plants. During the experiment it was supposed that in the series of *R. persicae* there may have been a latent case of blight in the healthy spinach from which the aphids had previously been taken in the field and that the virus had been carried from it to the experimental plants. The possibilities of seed or soil infection were eliminated, as thousands of plants were growing at the time under insect-free conditions though in soil known to have grown diseased spinach. No cases of blight developed on any of these plants.

The control plants in all of the cages were free from blight; therefore it was thought either that lettuce was an alternate host of the inciting factor of the disease or that in some unknown manner the plants became infected during the manipulation incidental to the transference of the aphids.

In order to check these points, a duplication was made of the transfers of the *Macrosiphum solanifolii* from lettuce to spinach. In this series 20 spinach plants were used, and 10 adult aphids born and reared on lettuce were placed on each plant, as in the original series. Every precaution was taken to prevent the infection of the plants in any other manner than through the agency of the aphids. Twenty additional plants used as controls were untreated. Twenty plants were inoculated with the juice obtained by crushing the lettuce leaves from which the aphids had been taken. Four cases of blight developed in 22, 22, 29, and 36 days, respectively, after inoculation among the plants upon which the aphids had been allowed to feed. All the untreated plants remained healthy, as did those which were prick-inoculated with the juice of lettuce leaves. Twenty control plants pricked with a flamed needle remained healthy.

Twenty healthy spinach plants were inoculated with the juice of crushed *Macrosiphum solanifolii* taken from the original lettuce plants. Ten plants were pricked with a sterile needle and used as controls. Two of the inoculated plants developed positive symptoms of the disease in 22 and 26 days, respectively. The 18 other plants in the series, together with the control plants, remained healthy until the experiment closed, 54 days later. Another series of inoculations was made with the juices of aphids which had been feeding on lettuce, eggplant (*Solanum melongena*), and peppers (*Capsicum* spp.), to inoculate healthy spinach plants.

In all cases *M. solanifolii* was used. The parent aphids were collected in the field from lettuce plants, brought to the greenhouse, and placed on the various food plants, on which they were allowed to remain until the first-generation offspring had been produced. The inoculations were made about the time the majority of the first generation had reached the fourth instar. The following results were obtained.

Forty spinach plants inoculated with the juice of aphids collected from lettuce gave one positive infection, and the plant developed typical blight. Twenty plants were inoculated with the juice of aphids reared on eggplants; two positive infections resulted. Twenty plants inoculated with the juice of aphids reared on peppers remained healthy. Forty plants were pricked with a sterile needle and remained healthy. Three lots of 10 plants each were inoculated with the juice of crushed lettuce leaves, eggplant leaves, and pepper leaves. These plants remained healthy.

The results obtained in these series indicate the improbability of lettuce or eggplant serving as alternate hosts of the virus. The possibility of experimental error or outside infection was also rendered unlikely through the various duplications of the work. The fact remains that aphids which had never come in contact with spinach produced a small percentage of infections when placed on healthy spinach plants; also, the juice of aphids similarly treated produced the disease in a few cases when inoculated into healthy plants.

NATURAL INFECTION OF SPINACH PLANTS

NATURAL INFECTION OF GREENHOUSE SEEDLINGS BY VIRUS-BEARING APHIDS

Blighted spinach plants were collected, transplanted to a bench in the greenhouse, and covered with a large glass cage. Some American-grown spinach seed which had been soaked for a few minutes in 1 to 100 formaldehyde was planted in this cage. Ten days after transplanting the blighted plants to the greenhouse cage it was observed that they were dying and that the aphids from these plants had crawled to the seedling plants which had come up about six days after planting the seed. Only the cotyledon leaves had developed on the seedling plants at that time. The aphids in this cage were killed by fumigation a short time thereafter and the transplanted blighted plants died. About 30 days after the seedlings had come up it was observed that some of them had developed characteristic symptoms of blight, and within the next 10 days the majority of the plants had developed striking symptoms. This included some 25 plants in all. About 40 days after the seedlings had come up, the aphids in this case having been killed by fumigation, a pot of 52 seedling plants was set in this cage among the blighted plants. Thirty-five days later none of the seedlings in this pot had developed any symptoms of blight, although they were surrounded by blighted plants. This indicates that blight is not transferred except by aphids or some mechanical means. After making this observation, individuals of *Rhopalosiphum persicae* known to be free from infection were transferred to this cage. Twenty-nine days later the majority of the plants in the pot of healthy seedlings previously transferred to this cage had developed characteristic symptoms of blight, thus indicating that the aphids had traveled and carried infection from the blighted to the healthy plants in the pot.

A number of *R. persicae* from blighted plants in the above cage were transferred to large plants in a field cage. Similar plants in the large cage were used as controls. Thirty-one days after they had been inoculated all of the plants had developed characteristic symptoms of blight, while the control plants remained healthy. These results substantiate those of previous experiments to the effect that spinach-blight is a specific disease which may be produced either in the field or in the greenhouse,

and may be readily transferred from the field to the greenhouse, and vice versa, by means of the virus causing this disease.

Blighted plants from the Station field were transplanted to pots of greenhouse soil and placed in cages. About these were placed pots of seedling spinach. Care was used that the leaves of the seedlings did not come in contact with the blighted plants. The next day it was observed that aphids had crawled from the blighted plants to the seedlings. Thirteen days later definite symptoms of blight were observed on these plants. These data indicate that aphids on the blighted field plants served as carriers of the blight to the seedlings and that the spinach-blight in the Station field was the same as that which had appeared in the fields on other farms.

To prove that the aphids serve as carriers of the virus and not as the cause of the blight, mottled leaves from the smaller of the above transplanted, blighted, field plants were used to inoculate four pots of spinach seedlings by mashing the blighted tissues into the leaves of the seedlings. Two similar pots of seedlings were pricked with a flamed needle to serve as controls. Eight days after inoculation seedling plants in all four of the above pots had developed mottled leaves characteristic of blight, especially about the points of inoculation. Thirteen days after inoculation the majority of these plants had developed typical symptoms of blight. Twenty-six days after inoculation practically all of the inoculated plants were in advanced stages of spinach-blight, while the controls remained healthy.

Blighted plants were collected from the Station field and brought to the laboratory, where aphids were allowed to crawl from them to a pot of 50 or more healthy seedlings. The next day it was observed that numerous aphids were present on the seedlings. Therefore the blighted plants were removed from the cage. Twenty-one days after inoculation some of the plants in this pot of seedlings had developed the mottled leaves characteristic of blight. Five days later a majority of the 50 or more plants developed typical symptoms of blight. The aphids originally transferred to this pot were killed by fumigation 31 days after inoculation. *Rhopalosiphum persicae* known to be free from infection were transferred to these plants. Numerous seedling plants were coming up in this pot from seed planted after the original aphids had been killed.

The majority of these secondary seedlings eventually blighted, thus indicating that the aphids had become virus bearers and had transferred the inciting entity to the seedlings.

Ten days after transferring the *Rhopalosiphum persicae* to the above pot of seedlings, a blighted leaf bearing both larval and adult aphids was removed from one of the plants and placed on a large plant in a field cage. Numerous spinach seedlings from seed planted somewhat later were growing about this plant. Thirty-two days after inoculation the large plants and two of the seedlings had developed positive symptoms

of blight. The plants left as controls appeared healthy. These results indicate that spinach-blight is caused by a definite virus, and is transmitted from plant to plant by the aphids.

COMPARISON OF DISEASED MATERIAL FROM VARIOUS LOCAL SOURCES

FARM C.—Diseased plants collected in two blighted areas on farm C were placed in a field cage, care being taken that the blighted plants did not touch the healthy spinach seedlings in the cage. Both species of aphids were present on the blighted plants. Sixty-one days later two of the seedling plants had developed the mottled leaves typical of spinach-blight. Twelve days later a third seedling plant developed definite symptoms of blight.

These results indicate that the aphids on introduced, blighted plants had crawled to the seedling plants and had infected them with the spinach-blight virus.

In order to prove the infection, one mottled leaf was removed from each of two diseased plants for prick inoculations. With a flamed needle the leaves of two spinach plants were pricked to serve as controls. Seven plants in two pots were inoculated by pricking through the mottled leaf of plant 1 into the healthy leaf of the seedling. Seven similar plants in two pots were inoculated by pricking into them the juice of the mottled leaf from plant 2. Twelve days after inoculation five plants developed typical symptoms of blight. Thirty-six days after inoculation 12 of the 14 inoculated plants had developed the mottled leaves characteristic of blight, but the control plants were large and apparently healthy. The other inoculated plants, although not showing typical symptoms of blight, did not have the healthy appearance of the control plants. These results indicate that the aphids are carriers of the virus, and that spinach-blight is caused by a specific virus which can be transferred from plant to plant by needle pricks as well as by aphids.

STATION FARM.—Blighted plants were collected from the Station field and piled on the ground in the edge of the woods near the greenhouse. Pots of spinach seedlings from seed from six different sources were placed in wire cages so as to have seed lots 1, 2, and 3 in one cage and 4, 5, and 6 in another. Two such series were made, a total of 12 pots in four cages. Some of the blighted plants from the above-mentioned pile were placed in each cage, care being exercised that the blighted plants did not touch the seedlings in the pots. The next day it was observed that *Macrosiphum solanifolii* and *Rhopalosiphum persicae* had crawled from the leaves of the blighted plants onto the leaves of the seedling plants; therefore the blighted plants were carefully removed from the cages so that they did not touch the seedlings. Six days later seedlings in seed series 1 and 5 had the mottled leaves characteristic of blight, and 11 days later a large percentage of the seedlings in each of the seed

series showed mottled leaves; but the controls remained healthy. These pots were kept under observation for the next month, and during this time the majority of the seedlings in all of the seed lots became mottled and stunted in growth, thus indicating that it made little difference as to the source of seed of the Savoy strain of spinach grown from commercial seed as regards the resistance to blight.

FARM D.—Blighted plants were collected from two fields on farm D and aphids from some of these plants were placed on a large pot containing about 100 spinach plants 13 days old. A similar pot of seedlings served as a control. Eighteen days after inoculation some of these seedling plants had developed the mottled leaves typical of blight. Twenty-nine days later some of the blighted plants were alive, but in advanced stages of the disease. Control plants remained healthy. These results indicate that diseased plants scattered over fields are caused by the same virus which produces large areas of blighted plants.

FARM B.—Blighted plants were collected from farm B and brought to the Station, where a number of *Rhopalosiphum persicae* and *Macrosiphum solanifolii* were removed from them and placed on healthy spinach plants growing in a field cage. The blighted plants were then placed in another field cage, care being taken not to allow them to come in contact with the seedlings nor to scatter any of the remaining aphids on the seedlings. A similar cage of field plants served as a control. Sixty-three days after inoculation, blighted plants were observed in both of the above cages, while the control plants remained healthy. These results indicate that the aphids served as carriers of the blight virus, it making little difference whether they were placed directly on the healthy plants or allowed to travel by themselves from the blighted plants to the healthy seedlings. Mottled leaves were removed from blighted plants 22 days after the disease had been first observed in the cages, and seven plants growing in two pots in the greenhouse were inoculated by mashing the diseased tissues into the healthy leaves of the seedlings. A similar pot of plants pricked with a flamed needle served as a control. Eight days after inoculation two of the plants had developed the mottled leaves characteristic of blight, while the five others appeared doubtful. Thirty-eight days after inoculation all seven of the plants had developed typical symptoms of blight, but the control plants remained healthy.

Similar results were obtained by inoculating potted plants in the greenhouse with a leaf from a blighted plant from the other field cage. These results indicate that the aphids carried the blight virus from the original field plants to the caged plants and that this virus was readily carried from a blighted, caged plant to the greenhouse plants by needle pricks.

FARM E.—On October 21, 1916, spinach seed was planted on a field where spinach had not been grown for some time. A characteristic large blighted area subsequently developed at one side of this field. On Janu-

ary 26, 1917, this area was photographed (Pl. 9, A). According to information obtained from the grower, spinach-blight began to develop in this area during the first week in January. Besides this large area, a few smaller areas also developed in the same field. Blighted plants were collected from the largest area and brought to the greenhouse, where individuals of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were removed and placed on six pots of spinach seedlings 1 month old. Similar plants were kept in another cage as controls. Nineteen days after inoculation the majority of the plants in each of the pots had developed characteristic symptoms of blight. Some of the plants from which the aphids had been removed were placed in a cage with six pots of spinach seedlings, care being taken that the blighted plants did not come in contact with the seedlings. The next day it was observed that many of the aphids had crawled to the seedling plants. Nineteen days after inoculation numerous blighted plants were observed among the pots of seedlings. The controls appeared healthy. These results indicate that the blighted plants on farm E were produced by the same specific virus which caused blight on other farms.

A few of the central leaves were removed from blighted field plants from which the aphids had been taken, and six pots of seedlings were inoculated by mashing the blighted tissue into the leaves. Seventeen days after inoculation, some of the plants in each of the above pots had mottled leaves. Forty days after inoculation plants in each of the pots were in the advanced stages of blight, while the control plants appeared healthy.

Forty-three days after inoculation a mottled leaf was removed from one of the blighted plants, and five healthy seedlings in another pot were inoculated by mashing the diseased tissues into them. Eighteen days later two of the plants had developed the mottled leaves of blight. The control plants appeared healthy.

With another mottled leaf, removed from a blighted plant 43 days after inoculation, five large plants in a field cage were inoculated by mashing the diseased tissues into the healthy plants. Similar plants in the same cage were pricked with a flamed needle and served as controls. Seventeen days after inoculation all five of the above plants had developed decidedly mottled leaves, but the control plants appeared healthy. These results show that the spinach-blight on this farm is due to a specific virus which may be transferred from diseased to healthy plants either by aphids or by needle pricks, thus indicating that it is the same disease as that occurring on other farms.

FARM F.—In a field of spring spinach on farm F located at least 10 miles from the Experiment Station a few scattered plants appeared to be affected with blight. Some of these were collected and brought to the greenhouse, where aphids were removed and placed on two pots of spinach seedlings. Similar pots of plants were used as controls. *Macro-*

siphum solanifolii were more abundant than *Rhopalosiphum persicae*. Two other pots of seedlings were inoculated by mashing diseased tissues from the blighted plants into the leaves of the seedlings. Nineteen days after inoculation one of the inoculated plants had developed characteristic symptoms of the blight. One mottled leaf was removed from this plant, and with it three seedlings in another pot were inoculated by mashing the diseased tissues into their leaves. With another leaf from the blighted plant inoculations were made in one of the field cages by mashing the blighted tissues into the leaves of the plants. Twenty-five days after the inoculation typical symptoms of blight appeared on eight plants in the field cage, while the control plants remained healthy. Farm F is located a considerable distance from the majority of the farms where the blighted plants used in the other experiments had been obtained. The fact that virus from the diseased plants on this farm produced blight in both greenhouse and field-grown plants indicates that the disease is the same as that occurring on the other farms.

COMPARISON OF SCATTERED, INDIVIDUAL BLIGHTED PLANTS WITH BLIGHTED PLANTS FROM AREAS

Further experiments were begun with the object of determining any variation in the nature and transmissibility of spinach-blight occurring in individually diseased plants and plants in diseased areas. Both species of aphids were used to make the transfers, and the material was checked each time by needle-prick inoculations. When transfers were made, an average of four insects were placed on each plant. Twenty-four hours later the plants were fumigated to kill the aphids. The healthy plants were 24 days old at the time of inoculation. The diseased material used for inoculation purposes was collected on various farms in the spinach-growing region of eastern Virginia. The results of this experiment are given in Table III. These show that the percentages of infection are similar for the individually diseased plants and for those collected in the diseased areas. This indicates that the factors producing the disease from both of the above sources are the same.

Since spinach-blight occurs both in scattered plants in the field and in distinct areas, it led to a study of the cause of these variations. Briefly stated, the results of this study indicated that under certain conditions favorable to the development of aphids there often occur heavy local infestations. During such periods the apterous females are particularly numerous and active, passing readily from plant to plant. Provided the favorable conditions persist, the infested area may be enlarged from a few plants to an area many yards in diameter. If a blighted plant occurs near the center of infestation, infection may be carried by the aphids to numerous other plants. When the conditions change and the aphids are greatly reduced in numbers, the spread of infection is likewise checked. Thus is an area of blighted plants formed. The infec-

tions in the individually diseased plants which occur scattered through out the fields are probably produced by the alate female aphids which fly from blighted plants carrying the infection to healthy plants upon which they alight and feed. These scattered, blighted plants in turn may serve as centers of infection for future diseased areas.

TABLE III.—*Transmission of spinach-blight from various local sources, Norfolk, Va., 1917*

Species of aphid used.	Source of diseased material.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.	Number of insects per plant.
				Days.	
Macrosiphum solanifolii...	Farm A (center of diseased area).	10	9	16	4
Do.....	Farm B (center of diseased area).	10	10	17	4
Do.....	Farm C (center of diseased area).	10	8	14.3	4
Do.....	Station farm (center of diseased area).	10	9	16.2	4
Rhopalosiphum persicae...	Farm A (center of diseased area).	10	6	17	4
Do.....	Farm B (center of diseased area).	10	8	17.7	4
Do.....	Station farm (center of diseased area).	10	9	16	4
Macrosiphum solanifolii...	Farm A (individually diseased plant).	10	8	16.4	4
Do.....	Farm B (individually diseased plant).	10	8	14	4
Do.....	Station farm (individually diseased plant).	10	9	17.3	4
Rhopalosiphum persicae...	Farm A (individually diseased plant).	10	6	19.2	4
Do.....	Farm B (individually diseased plant).	10	7	16.1	4
Controls.....	Untreated, 20, all healthy.....	.....	.....	.....	.....

EXPERIMENTS ON THE INSECT TRANSMISSION OF SPINACH-BLIGHT, 1916–17

RELATIVE INFECTIVITY OF APHIDS OBTAINED FROM PLANTS IN VARIOUS STAGES OF THE DISEASE

A series of experiments was begun on January 12, 1917, to determine the relative infectivity of the virus from plants in various stages of spinach-blight. As has been discussed, there are eight arbitrary but rather definite stages of advancement of the disease between the first appearance of pathogenic symptoms and the ultimate death of the plant. In order to make this test, it was necessary to collect typically diseased field plants in the various stages. A large number of lettuce-fed aphids were placed on the diseased plants and allowed to feed for three days before transferring them to healthy spinach plants. Both species of

aphids were used in the transfers, and inoculations were also made with the virus obtained from the blighted plants. The first to sixth stages, inclusive, were used. Plants in the seventh and eighth stages are more or less dried, withered, and rarely serve as food for aphids in the field. Ten aphids from diseased plants were transferred in each case and remained on the healthy plants for 48 hours.

TABLE IV.—*Relative infectivity of aphids obtained from plants in various stages of spinach-blight at Norfolk, Va., 1917*

Source of material used in making inoculations.	Number of plants inoculated by the transference of insects.	Number of infections.	Average length of period of incubation.	Number of plants inoculated with virus.	Number of infections produced.	Average length of period of incubation.
			Days.			*Days.*
Plant in sixth stage of disease	4	4	13	2	2	15
Plant in fifth stage of disease	4	4	14.5	2	1	17
Plant in fourth stage of disease	4	4	14	2	2	19
Plant in third stage of disease	4	4	19	2	1	21
Plant in second stage of disease	4	4	22	2	2	22.5
Plant in first stage of disease	4	2	25.5	2	1	30
Healthy plant	4	0	0	2	0	0

RELATION BETWEEN LENGTH OF INOCULATION PERIOD AND NUMBER OF INFECTIONS PRODUCED

From Table IV it will be seen that the ratio between the inoculations and infections obtained were about the same for all the stages between the second and sixth, inclusive, which would indicate that the virus from the various stages is approximately equally infectious. The most striking results obtained were the variation in the incubation period of the disease in the inoculated plants. The incubation period in the plants inoculated by insects transferred from the sixth-stage-diseased plants averaged 13 days and in the plants inoculated directly with the virus by needle pricks it was 15 days. For plants inoculated by insects from the fifth-stage-diseased plant the incubation period of the disease was 14.5 days. Direct needle-prick inoculations with the virus from the same source resulted in an average incubation period of 17 days. The incubation period gradually increased in length with the fourth, third, and second stages until in the first stage it had reached a length of 25.5 days before the disease was produced by insects and of 30 days before the disease was produced by direct needle-prick inoculation with the virus. These figures indicate that, while the virus from the various stages may be about equally infectious, so far as the ability to produce the disease is concerned, yet owing perhaps to the concentration of the infective entity in the plant juices, which increases as the disease advances, the virus of the more

advanced stages of the blight produced positive symptoms in about half the time that was required to produce them, when plants were inoculated with the virus obtained from plants in the early stages of the disease. It is also worthy of note that under conditions present in this experiment, when 10 adult aphids were transferred from a diseased to a healthy plant, positive symptoms of the disease developed in two to five days' less time than when the inoculation was made with the same diseased material by means of the expressed virus pricked into the plants with a sterile needle. No infections were obtained in the transfers of lettuce-fed aphids to healthy spinach, and no infections were obtained when prick inoculations were made with the juice obtained from healthy spinach plants.

In order to determine the approximate length of time which aphids from blighted spinach must remain on healthy plants to produce infections, aphids from diseased plants were transferred to a series of healthy plants and allowed to remain on them for various periods of time. Since infections were previously obtained by allowing the insects to remain on plants for 48 hours, the length of the periods in the present series were shortened to 5 minutes, 2 hours, 14 hours, and 24 hours. As the aphids had been disturbed, they fed little during the 5-minute period that they were allowed to remain on the healthy plants. They were observed occasionally to plunge their beaks into the plant tissues for an instant, quickly withdrawing them, moving to some other part of the plant and repeating the performance. The results of this experiment are given in Table V.

TABLE V.—*Results of experiments on the relation between the length of the inoculation period and the number of infections produced*

Length of time aphids remained on the plants.	Species used or treatment.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.	Number of plants remaining healthy.
				Days.	
24 hours	*Macrosiphum solanifolii*	14	11	15.2	3
14 hours	do	19	15	18.8	4
2 hours	do	9	6	16.3	3
5 minutes	do	8	4	24	4
24 hours	*Rhopalosiphum persicae*	17	12	16.3	5
14 hours	do	13	8	15	5
2 hours	do	8	5	14	3
5 minutes	do	9	2	27.5	7
	Plants inoculated with virus from the original diseased plant	10	8	16.1	2
Controls	Untreated	20			20

Where the aphids remained on the plants for only 5 minutes less infections were obtained than when they fed on the plants for 2 hours or longer. The percentage of infections which resulted from the longer

periods of inoculation varied from 50 to 75 per cent, but apparently had no direct relationship with the length of time the aphids had been on the plant. Little difference could be observed in the infectivity of the two species. The length of the incubation period of the blight showed but slight variation in the various plants affected, 14 to 18.8 days, and there was no indication of a relationship between its length and the length of the inoculation period. The shortest average incubation period was obtained with individuals of *Rhopalosiphum persicae* placed on the plants for 2 hours, while the longest was when *Macrosiphum solanifolii* were placed on the plants for 14 hours. When a number of *M. solanifolii* were allowed to remain on the plants for 5 minutes 4 plants out of 8 became infected and showed positive symptoms on the twenty-fourth day. Where *R. persicae* were used, 2 plants out of 9 showed positive symptoms in an average time of 27.5 days. Of 10 plants inoculated with the virus of the original diseased plant from which the aphids were taken, 8 became infected and developed positive blight symptoms in an average time of 16.1 days. Ten untreated plants used as controls remained healthy; hence, it will be seen that virus-bearing aphids produce infections when they feed on healthy plants for only a few minutes.

INFECTIVITY OF MATURE AND IMMATURE APHIDS

The following series of transfers were made in order to determine the relative infectivity of aphids in their various stages of development. These were performed with both *Macrosiphum solanifolii* and *Rhopalosiphum persicae*. The insects were reared and allowed to feed, reproduce, and develop on typical fourth-stage-diseased spinach plants. Individuals representing each of the five instars were transferred to series of healthy plants. Transfers were also made with adult alate and apterous females. A series of 10 plants were inoculated with insects in each instar, four insects to each plant. They were allowed to remain on the plants for a period of 48 hours. As will be seen from Table VI, those individuals of *M. solanifolii* of the first instar which had been transferred directly from the diseased to the healthy plants produced 1 infection in 10 inoculations, positive symptoms appearing on the twenty-second day (Pl. 9, B). The remaining plants were healthy when the experiment closed, 54 days later. The second instar of *M. solanifolii* produced 4 infections out of 10 inoculations, positive symptoms appearing in an average time of 19 days. The third instar of *M. solanifolii* produced 4 infections in an average time of 18.5 days. The fourth instar of *M. solanifolii* produced 8 infections in an average time of 17.6 days. The fifth instar, alate form, produced 9 infections in 17.2 days, while the fifth instar, apterous form, produced 7 infections in an average time of 14 days. It will be seen that when healthy plants are inoculated by transferring to them adult aphids from diseased plants, the incubation period of the disease is several days shorter than it is where aphids in

the first or second instars are used. The percentage of infections also is increased according to the age of the aphids when they are transferred from disease to healthy plants.

TABLE VI.—*Infectivity of mature and immature aphids*

Species.	Instar.	Number of insects to plant.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.	Number of plants remaining healthy.
					Days.	
Macrosiphum solanifolii.....	First.....	4	10	1	22	9
Do.....	Second.....	4	10	4	19	6
Do.....	Third.....	4	10	4	18.5	6
Do.....	Fourth.....	4	10	8	17.6	2
Do.....	Fifth alate.....	4	10	9	17.2	1
Do.....	Fifth apterous.....	4	10	7	14	3
Rhopalosiphum persicae.....	First.....	4	10	.....	.....	10
Do.....	Second.....	4	10	3	18.2	7
Do.....	Third.....	4	10	4	19	6
Do.....	Fourth.....	4	10	6	16.3	4
Do.....	Fifth alate.....	4	10	7	18	3
Do.....	Fifth apterous.....	4	10	8	15.5	2

The results obtained with *Rhopalosiphum persicae* were similar to those obtained with *Macrosiphum solanifolii*, especially in regard to the increased number of infections obtained with mature aphids over those resulting from the transfers of the immature stages. The length of the incubation period of the disease is also decreased where the older aphids are used. No marked differences could be observed in the ability of the two species to produce infections.

ABILITY OF APHIDS TO CARRY INFECTION TO MORE THAN ONE HEALTHY PLANT

A series of experiments were started to determine whether an aphid can carry the virus of the disease to more than one healthy plant after leaving the original diseased plant. Three series of inoculations were made. In the first series the insects were allowed to remain on the plants for 24 hours; in the second, 14 hours; and in the third, 2 hours. The diseased plants from which the aphids were obtained were carefully checked out by means of prick inoculations with virus, and positive proof of their being affected with the disease was thus obtained. The healthy plants used in the inoculations were 4 weeks old at the time the aphids were placed on them. Three or four plants were allowed to grow in 4-inch pots and the aphids were placed on these for each period. This gave a record of several plants in each series. The results of this experiment are given in Table VII. In comparing the results obtained with the transfers for the several periods it will be seen that where the insects remained on the plants for 24 hours the incubation periods were

shorter than when they remained on the plants for either 2 or 14 hours. There was apparently no greater number of infections obtained in the 24-hour period than in either of the others. These data prove that aphids have the ability to transmit spinach-blight to several plants after leaving a diseased host. In this manner alate forms flying from one healthy plant to another after leaving the blighted spinach might infect a large number of plants within a comparatively short time, and will perhaps explain the sudden and widespread appearance of blight which usually occurs after an outbreak of aphids, when many alate individuals are produced.

TABLE VII.—*Ability of aphids to carry infections to more than one healthy plant*

Plant No.	Length of time aphids remained on plants.	Number of plants.	Number of plants infected.	Average length of incubation period.	Number of insects per plant.	Number of plants remaining healthy.
	Hours.			*Days.*		
1	24	4	4	15	2	1
2	24	4	3	17	2	1
3	24	4	2	25	2	2
4	24	4	1	34	2	3
5	24	4	2	30	2	2
1	14	3	3	19	2	0
2	14	4	2	21	2	2
3	14	3	1	28	2	3
4	14	4	0	0	2	4
5	14	3	1	31	2	2
1	2	4	4	22	2	0
2	2	4	3	28	2	1
3	2	4	2	31	2	2
4	2	4	1	30	2	1
5	2	4	1	34	2	3

INOCULATIONS WITH THE JUICE OBTAINED BY CRUSHING APHIDS COLLECTED ON BLIGHTED SPINACH

As a check on the data collected in other experiments—namely, that the juice of crushed aphids from blighted plants is virulent—the following series was started. Several healthy plants were inoculated with the virus by means of needle pricks. When the plants had developed typical symptoms of blight, a large number of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were placed upon them and allowed to feed for a period of 20 days. A sufficient number of adults were collected from these plants and crushed, about 1 c. c. of juice being thus obtained. A series of healthy plants 28 days old was inoculated with this juice. Of 42 plants inoculated with juice of crushed *M. solanifolii*, 27 became infected, positive symptoms appearing in an average time of 23.1 days. Thirty-eight plants were inoculated with the juice of crushed *R. persicae*,

and 21 infections were produced. The symptoms appeared in an average time of 26.4 days. From these data it is evident that the juice obtained by crushing virus-bearing aphids is virulent.

TRANSFERS OF STRAINS OF APHIDS OBTAINED FROM OTHER STATES IN COMPARISON WITH TRANSFERS OF LOCAL APHIDS WHICH WERE SUPPOSED NOT TO BE CARRYING THE SPINACH-BLIGHT VIRUS

In some of the earlier experiments infections were produced when aphids which were supposedly not virus carriers were transferred to healthy plants. These led to the following experiment the object of which was to determine, so far as possible, the conditions which cause aphids to become virus carriers. In order to accomplish this, it was necessary to obtain strains of aphids from localities where, so far as is known, spinach-blight does not occur. Through the kindness of Dr. W. E. Hinds, Entomologist, Alabama Agricultural Experiment Station, a supply of *Macrosiphum solanifolii* on lettuce was obtained from Auburn, Ala. From Mr. Thomas H. Jones, Entomological Assistant, Truck Crop Insect Investigations, United States Bureau of Entomology, was obtained a large supply of *Rhopalosiphum persicae* collected on peppers in a greenhouse at Baton Rouge, La. Prof. C. P. Gillette and Mr. L. C. Bragg, of the Colorado Experiment Station, furnished us with a splendid lot of eggs of *R. persicae* on peach twigs from Fort Collins, Col. Prof. J. R. Watson, Entomologist, Florida Agricultural Experiment Station, furnished several live individuals of *R. persicae*, among other species collected from *Hibiscus sabdadriffa*.

As the various lots of aphids were received, they were placed, with the exception of a few individuals used immediately for experimental purposes, in cages on lettuce, eggplants, and healthy spinach. The aphids were allowed to feed and reproduce for several generations on the food plants mentioned until many thousand individuals of each strain were thus obtained. The eggs of *Rhopalosiphum persicae* were hatching at the time they arrived. The young stem mothers remained on the peach twigs until three generations of offspring were produced. The fourth generation was transferred to lettuce and healthy spinach. In this manner were obtained a sufficient number of aphids from widely separated regions, for use in comparison with local aphids collected in the vicinity of Norfolk, Va. Collections of local adult *Macrosiphum solanifolii* and *R. persicae* were made from diseased plants in the field. These were placed in cages on lettuce seedlings where they were allowed to feed and reproduce for five days. At the end of this period the adults were removed and destroyed. Some of the offspring were placed on other lettuce seedlings, some on eggplants, and the remainder on healthy spinach seedlings grown under insect-free conditions. The third week after the local aphids had been placed on the spinach seedlings some of these plants showed evidence of being diseased. Evidently they became

infected through the agency of the aphids placed upon them. The insects were confined on these plants for about five weeks during which time approximately four generations of first-born young were produced. This gave a large supply of supposedly nonvirus-carrying local aphids for use in making the transfers to healthy plants, and these insects and their offspring are referred to hereafter as the "Norfolk, Va., strain." The mean temperature in the cages in which the aphids were confined was 76° F., with a relative humidity varying from 55 to 95 per cent. The results of the transfers are given in Table VIII.

TABLE VIII.—*Transfers of strains of supposedly non-virus-bearing aphids obtained from various States in comparison with the transfers of the local (Norfolk, Va.) supposedly non-virus-bearing strain*

Species used.	Source of insect.	Previous food plant of insect.	Number of plants inoculated.	Length of time insects were on plant.	Number of plants infected.	Average length of incubation.	Number of insects placed on each plant.
				Hours.		*Days.*	
Macrosiphum solanifolii.	Norfolk, Va.	Lettuce	76	48	2	28	5
Rhopalosiphum persicae.	do	do	52	48	3	27.3	5
Macrosiphum solanifolii.	do	Eggplant	24	48	2	31	2
Rhopalosiphum persicae.	do	do	27	48	1	34	2
Macrosiphum solanifolii.	do	Spinach	[a]500	48	39	28.4	[a]2
Rhopalosiphum persicae.	do	do	[a]500	48	27	32.1	[a]2
Macrosiphum solanifolii.	Auburn, Ala.	Lettuce	100	48	0	.....	2
Do	do	Spinach	100	48	0	.....	2
Do	do	Eggplant	20	48	0	.....	2
Rhopalosiphum persicae.	Baton Rouge, La.	Pepper	50	48	0	.....	2
Do	do	Spinach	100	48	0	.....	2
Do	Fort Collins, Colo.	Peach	20	48	0	.....	2
Do	do	Spinach	50	48	0	.....	2
Do	Gainesville, Fla.	do	40	48	0	.....	2

[a] Approximately.

To 76 healthy spinach plants were transferred 400 of *Macrosiphum solanifolii* (Norfolk, Va., strain) which had previously been feeding on lettuce. The insects remained on the plants for 48 hours. Two of the spinach seedlings developed positive symptoms of blight in an average time of 28 days. About 250 individuals of *Rhopalosiphum persicae* (Norfolk, Va., strain) which had previously been feeding on lettuce were transferred to 52 healthy spinach seedlings, on which they were allowed to feed for 48 hours. Three of the spinach plants developed symptoms of blight in an average time of 27.3 days. Fifty of *Macrosiphum solanifolii* (Nor-

folk, Va., strain) were transferred from eggplant to 24 healthy spinach seedlings. Two of the plants developed positive symptoms of blight in 30 and 33 days, respectively. Fifty of *Rhopalosiphum persicae* (Norfolk, Va., strain) which had been feeding on eggplant were transferred to 27 healthy spinach seedlings. One infection resulted, and the first positive symptoms developed on the thirty-fourth day.

In one of the large field cages seed was sown broadcast, and about 500 healthy plants were thus obtained. One thousand individuals of *Macrosiphum solanifolii* (Norfolk, Va., strain) which had previously been feeding on spinach were transferred to the plants in this cage. At the end of of the 48-hour period that the aphids were allowed to remain on the plants, the cages were fumigated on three consecutive days with nicotine. In this way all the aphids were destroyed. Thirty-nine cases of blight developed on the plants in an average time of 28.4 days. About 1,000 individuals of *Rhopalosiphum persicae* (Norfolk, Va., strain) were transferred to a second large field cage in which were growing approximately 500 spinach seedlings. After the aphids had remained on the plants for 48 hours, they in turn were destroyed by three successive fumigations. Twenty-seven of the plants in this cage became infected with blight. The first positive symptoms appeared in an average time of 32.1 days. Two hundred of *Macrosiphum solanifolii* (Auburn, Ala., strain) from healthy spinach were transferred to a cage containing 100 healthy spinach seedlings. No infections were obtained. Forty of *Macrosiphum solanifolii* (Auburn, Ala., strain) were transferred from eggplant to 20 healthy spinach seedlings. No infections were obtained. In a similar manner the following transfers were made. The insects remained on the plants for 48 hours in each case before they were destroyed. One hundred of *Rhopalosiphum persicae* (Baton Rouge, La., strain) from peppers, were transferred to 50 healthy spinach seedlings. Two hundred of *Rhopalosiphum persicae* (Baton Rouge, La., strain) were transferred from healthy spinach to healthy spinach seedlings. Forty of *Rhopalosiphum persicae* (Fort Collins, Colo., strain) were transferred from peach to 20 healthy spinach seedlings. One hundred of *Rhopalosiphum persicae* (Fort Collins, Colo., strain) were transferred from healthy spinach to 50 healthy spinach seedlings. Eighty of *Rhopalosiphum persicae* (Gainesville, Fla., strain) were transferred from healthy spinach to 40 healthy seedlings. No infections were obtained as a result of any of the above transfers, and the seedling plants remained healthy in every case until the close of the experiments.

A series of healthy spinach plants were inoculated with the juice of the lettuce, eggplant, spinach, and pepper plants, upon which the aphids had been feeding previous to their transference to the healthy spinach seedlings in the experiment. No infections resulted from these inoculations, except from the inoculations with the juice of the spinach plants on which the Norfolk strain had been feeding, in which case 2 positive infections resulted from 10 inoculations.

A series of inoculations (see Table IX) were made, supplementing the transfers of the live insects. Aphids from the same sources as those used in the transfers were crushed and the juice thus obtained was inoculated into the tissues of the healthy plants by means of needle pricks. Fifty healthy plants were inoculated with the juice of *Macrosiphum solanifolii* (Norfolk, Va., strain) which had previously been feeding on lettuce. Four plants became infected, and symptoms of blight developed in an average time of 29.2 days. Fifty plants were inoculated with the juice of *M. solanifolii* (Norfolk, Va., strain) which had previously been feeding on spinach. Eight plants developed symptoms of blight in an average time of 31.6 days. Fifty plants were inoculated with the juice of *R. persicae* (Norfolk strain) which had previously been feeding on lettuce. One plant became infected. The first positive symptoms of the disease appeared in 36 days. Fifty plants were inoculated with the juice of *R. persicae* (Norfolk strain) which had previously been feeding on spinach. Four plants became infected in 31.9 days. In a similar manner healthy spinach was inoculated with the juice of *M. solanifolii* (Auburn, Ala., strain) from lettuce and healthy spinach. Healthy spinach was inoculated with the juice of *R. persicae* (Baton Rouge, La., strain) from pepper, healthy spinach, and lettuce. The juice obtained from crushed *R. persicae* (Fort Collins, Colo., strain) which had been feeding on peach and healthy spinach was inoculated into healthy spinach seedlings. All of these inoculations gave negative results, the plants remaining healthy until the experiment was closed, two months after the inoculations had been made.

TABLE IX.—*Results of inoculations with the juice of crushed, supposedly non-virus-bearing aphids*

Species used	Source of insect.	Previous food plants of insect.	Number of plants inoculated with the juice of crushed, supposedly non-virus-bearing aphids.	Number of plants infected.	Average length of incubation period.
					Days.
Macrosiphum solanifolii	Norfolk, Va.	Lettuce	50	4	29.2
Do	do	Spinach	50	8	31.6
Rhopalosiphum persicae	do	Lettuce	50	1	36
Do	do	Spinach	50	4	31.9
M. solanifolii	Auburn, Ala.	Lettuce	25	All healthy	.....
Do	do	Spinach	25	do	.....
R. persicae	Baton Rouge, La.	Pepper	25	do	.....
Do	do	Spinach	25	do	.....
Do	do	Lettuce	25	do	.....
Do	Fort Collins, Colo.	Peach	10	do	.....
Do	do	Spinach	25	do	.....

It is evident from the preceding data relative to aphids which have fed on blighted plants that a small percentage of their offspring, although these may have have been born and reared on plants other than spinach, may produce infections of blight when they are transferred to healthy spinach plants. Inoculations made with the juices of lettuce, eggplant, and supposedly healthy spinach produced no infections; hence, it is unlikely that either the lettuce or the eggplant served as alternate hosts for the inciting factor of the disease. The possibility that the blighted condition was due to a mechanical or other stimulus produced directly by the insect was disproved by the fact that aphids from the regions mentioned, where as yet the occurrence of spinach-blight has not been reported, were incapable of producing infections on healthy spinach unless they had fed on a diseased plant previous to their transference to a healthy plant. It was also found that the juice of Norfolk aphids, although born and reared on plants other than spinach, occasionally produced infections of blight when inoculated into healthy plants. The percentage of infection obtained by transferring the local strain of aphids from lettuce, eggplant, or peppers to healthy spinach plants was small. The transfers of local aphids from supposedly healthy spinach to known healthy spinach resulted in a larger number of infections than in cases where the aphids were transferred from lettuce or eggplant to healthy spinach. As it subsequently appeared, these supposedly healthy plants were diseased at the time the transfers were made from them to the known healthy plants. They became infected in all probability through the agency of the original aphids transferred from the lettuce seedlings. The plants were small and the aphids were numerous; therefore it was difficult to distinguish between the early visible symptoms of the disease and the somewhat yellowish condition caused by the attacks of numerous aphids. Subsequent inoculations proved that the juice taken from these plants was virulent; hence, it was a simple matter for infections to be carried from these to other plants when the transfers of insects were made. Inoculations made with the juices of lettuce, eggplant, and peppers used as food for the aphids before their transference to healthy spinach gave no indications of any infections.

In the light of these findings—namely, (1) that the offspring of local virus-bearing aphids, although born and reared on plants other than spinach, are capable under certain conditions of producing infections of blight in healthy spinach plants to which they have been transferred; (2) that the juices of the plants other than spinach upon which these aphids were reared were nonvirulent; (3) that aphids of foreign strains are incapable of producing the blight on healthy spinach, unless they have first fed on diseased plants—the assumption must be taken that, whatever the entity is which caused the pathological transformations in the growth and development of normal spinach plants, it must be in some manner transmitted from the parent aphids to their offspring, as the offspring in turn may cause infections in healthy plants upon which they feed.

LENGTH OF TIME NON-VIRUS-BEARING APHIDS MUST REMAIN ON DISEASED PLANTS BEFORE THEY BECOME VIRUS BEARERS

As it was found that the offspring of aphids from Alabama, Louisiana, Colorado, and Florida did not produce blight infections when reared on lettuce, eggplant, or healthy spinach unless they had first fed on a blighted spinach plant, the following experiments were performed to determine the length of time that these aphids must remain on a diseased plant before they become virus bearers. Offspring of *Macrosiphum solanifolii* from Alabama and *Rhopalosiphum persicae* from Louisiana were placed on blighted spinach plants. The disease had been produced by means of needle-prick inoculations and the pathogenicity had been proved before the aphids were transferred to them. The results are presented in Table X.

TABLE X.—*Length of time non-virus-bearing aphids must remain on diseased plants before they become virus carriers*

Species.	Length of time aphids remained on blighted plants.	Number of plants inoculated.	Number of plants infected.	Average period of incubation.
				Days.
Macrosiphum solanifolii (Alabama)	48 hours	10	9	18.1
Do	24 hours	10	8	17.2
Do	14 hours	10	10	18.7
Do	2 hours	10	6	21.3
Do	10 minutes	10	2	24
Control	48 hours	10	.....	.....
Rhopalosiphum persicae (Louisiana)	24 hours	10	7	17
Do	14 hours	10	6	17.5
Do	2 hours	10	4	20
Do	10 minutes	10	.....	.....
Control	48 hours	10	.....	.....

After the aphids had remained on the diseased plants for 10 minutes, 30 of *Macrosiphum solanifolii* (Auburn, Ala., strain) were removed and placed on ten 22-day-old spinach seedlings. The insects remained on the plants for 24 hours and were then killed by fumigation. Of the 10 plants thus inoculated 2 became infected, the positive symptoms appearing in an average time of 24 days. In a similar manner 10 healthy spinach seedlings were inoculated by transferring to them 30 of *Rhopalosiphum persicae* (Louisiana strain) which had previously been allowed to feed on the diseased plants for 10 minutes. No infections occurred. Thirty of *M. solanifolii* were removed after feeding on diseased plants for a period of two hours and placed on 10 healthy spinach seedlings. Six infections resulted, the positive symptoms appearing in an average time of 21.3 days. Ten plants were inoculated with *R. persicae* (Louisiana strain) which had been feeding on a diseased plant for two hours. Four plants became infected in an average time of 20 days. All of the 10 plants

inoculated with *M. solanifolii* which had been feeding on a diseased plant for a period of 14 hours were infected, the positive symptoms appearing in an average time of 18.7 days. Thirty of *R. persicae* which had been feeding on the diseased plant for 14 hours were transferred to 10 healthy plants and produced 6 infections. The symptoms appeared in an average time of 17.5 days. Similarly 30 of *M. solanifolii* which had been feeding on the diseased plant for 24 hours and 30 which had been feeding on the same plant for 48 hours were transferred to two lots of 10 healthy seedlings each. The aphids which fed on the diseased plants for 24 hours, produced 8 infections. The symptoms appeared in an average time of 17.2 days. The aphids which were allowed to remain on the plants for 48 hours produced 9 infections when transferred to the 10 healthy spinach seedlings. Positive symptoms appeared in the average time of 18.1 days. Thirty of *R. persicae* allowed to remain on the diseased plants for 24 hours, when transferred to 10 healthy spinach seedlings, produced 7 infections. The symptoms appeared in an average time of 17 days. As a check on the foregoing experiments, 30 of *M. solanifolii* and 30 of *R. persicae* were allowed to feed on healthy spinach plants for 48 hours and were then transferred to two lots of 10 plants each, on which they remained for 24 hours. No infections resulted.

The foregoing data indicate that a few aphids become virus bearers when they remain on diseased plants for 10 minutes. At the end of 14 hours the individuals of *M. solanifolii* reached the maximum in their capacity to transmit the virus, whereas individuals of *R. persicae* of the 24-hour group produced one more infection than did those which had been on the plants for only 14 hours. Probably under field conditions, where the insects are undisturbed, feeding takes place more readily than when they are more or less excited from artificial transfers, and they become virus bearers in much less time than experiments would indicate. According to findings reported elsewhere, when virus-bearing aphids are transferred to healthy plants, there is no appreciable relationship between the number of infections produced and the length of time the aphids remain on the healthy plants, although in the present instance there is a distinct relationship between the time the aphids which have not previously been associated with blighted plants remained on the diseased plant and the number of infections they subsequently produced. The longer the aphids remain on the diseased plants before being transferred, the shorter the time until visible symptoms of blight appeared on the inoculated seedlings.

DO IMMATURE APHIDS FROM DISEASED PLANTS BECOME NON-VIRUS-BEARERS AS A RESULT OF MOLTING?

A number of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* in the fourth instar were collected in the field from a diseased plant and placed in individual sterile vials for a period of 24 hours. During this time they

were given no food. The individuals which had molted and become adults in the vials were transferred to healthy plants. Forty of *M. solanifolii* were placed on 20 healthy spinach plants on which they were allowed to remain for 48 hours. Six of the plants developed positive symptoms of the disease in an average time of 24.3 days. Forty of *R. persicae* were transferred to 20 spinach plants for a period of 48 hours. Four plants became infected and the symptoms appeared in an average time of 25.2 days. From these results it appears that when molting occurs after an aphid has left a diseased plant the insect may even then be able to produce infections of blight in healthy plants upon which it feeds, and indicates that the virus is transmitted in some other manner than on the external appendages of the insect's body.

ABILITY OF OFFSPRING OF VIRUS-BEARING APHIDS TO CARRY INFECTION

A series of healthy plants were inoculated by transferring to them at the time of birth the offspring of virus-bearing aphids. The young aphids had neither been allowed to feed previous to their transference to healthy plants; nor had they in any way come in direct contact with spinach affected with the disease. A number of adult female virus-bearing aphids were transferred directly to healthy spinach plants. The offspring of these females were transferred to the experimental plants as soon as they were born. Fifty of the immature *Macrosiphum solanifolii* were transferred to 25 healthy spinach plants and allowed to remain on them for a period of four days. They were then destroyed by fumigation. One plant became infected, and positive symptoms of blight appeared on the twenty-eighth day. As the young aphids had not taken food previous to their transference nor had any association with diseased plants before being placed on the healthy seedlings, the infections obtained of blight indicated that the young became associated with the infectious entity previous to their birth.

TRANSMISSION OF THE INFECTIOUS ENTITY OF BLIGHT BY ADULT VIRUS-BEARING APHIDS TO THEIR OFFSPRING

The results obtained in the earlier experiments on the insect transmission of spinach-blight indicated the possibility that the inciting factor of the disease was transmissible by a parent aphid to its offspring, thereby rendering the latter capable of producing infections in healthy spinach, although they had not previously fed on a diseased plant. The following experiments were performed to obtain data relative to this point. Both species of aphids were used and consisted of the Virginia, Alabama, and Louisiana strains previously discussed. The strains were confined in separate cages and were pure. On the same day the various lots of aphids were placed on fourth-stage-diseased plants in which the disease had been produced by virus inoculations made 42 days previously. The

adult aphids fed on the spinach for 7 days and were then retransferred to lettuce. On these plants they remained for 3 days, or until about 200 first-generation young had been born to each lot. The adults were removed, some being transferred to healthy plants for 48 hours. These were termed the "original parent aphids." When the offspring reached maturity and had produced between 200 and 300 young, the first-generation adults were removed and, as before, some were transferred to healthy plants. In like manner, as the second, third, and fourth generations matured, they were transferred to series of healthy spinach plants. Thus, inoculations were obtained from four generations representing three strains and two species of aphids. Two separate tests were conducted with each species: one in the insectary on potted plants and another in the field cages on plants grown under outdoor conditions. Five insects were placed on each plant and 10, 20, or 25 plants were used with each generation in each series. The results of this experiment are given in Table XI.

TABLE XI.—*Transmission of the infectious entity of spinach-blight by virus-bearing aphids to their offspring*

Species used.	Generation.	Number of insects per plant.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.
					Days.
GREENHOUSE SERIES:					
Macrosiphum solanifolii (Norfolk, Va., strain).	Original parent aphids from diseased plants.	5	25	23	18.1
M. solanifolii	First	5	25	8	24.2
Do	Second	5	25	2	33
Do	Third	5	25	2	34
Do	Fourth	5	25	1	37
OUTDOOR SERIES:					
M. solanifolii	Original	5	10	10	22
Do	First	5	10	2	37
Do	Second	5	10	3	34.3
Do	Third	5	10	1	39
Do	Fourth	5	10	0	0
GREENHOUSE SERIES:					
Rhopalosiphum persicae (Norfolk, Va., strain).	Original virus-bearing aphids.	5	25	21	17.4
Do	First	5	25	4	24.2
Do	Second	5	25	2	26
Do	Third	5	25	1	24
Do	Fourth	5	25	0	0
OUTDOOR SERIES:					
R. persicae	Original	5	25	18	21.3
Do	First	5	25	2	28
Do	Second	5	25	1	37
Do	Third	5	25	2	32
Do	Fourth	5	25	1	35
GREENHOUSE SERIES:					
M. solanifolii Auburn, (Ala., strain).	Original	5	10	9	17
Do	First	5	10	2	24
Do	Second	5	10	Healthy.	.....
Do	Third	5	10	2	32
Do	Fourth	5	10	1	36

TABLE XI.—*Transmission of the infectious entity of spinach-blight by virus-bearing aphids to their offspring*—Continued.

Species used.	Generation.	Number of insects per plant.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.
OUTDOOR SERIES:					*Days.*
M. solanifolii (Auburn, Ala., strain).	Original	2	10	10	21.3
Do	First	2	10	Healthy.	.....
Do	Second	2	10	2	35
Do	Third	2	10	2	38
Do	Fourth	2	10	.....	.....
GREENHOUSE SERIES:					
R. persicae (Baton Rouge, La., strain).	Original	5	20	19	19
Do	First	5	20	2	28
Do	Second	5	20	Healthy.	.....
Do	Third	5	20	1	31
Do	Fourth	5	20	Healthy.	.....
OUTDOOR SERIES:					
R. persicae (Baton Rouge, La., strain).	Original	5	10	8	24
Do	First	5	10	3	27
Do	Second	5	10	1	36
Do	Third	5	10	2	35
Do	Fourth	5	10	.....	.....

The original virus-bearing Norfolk strain of *Macrosiphum solanifolii* gave high percentages of infection both on the insectary and field plants. One hundred per cent of these plants became infected in the field cages; this was unusual, considering that the aphids had fed on lettuce for several days between the time they were taken from diseased and the time they were placed on the healthy spinach plants. Eight infections out of 25 inoculations, or 32 per cent, occurred on those inoculated in the insectary with the first-generation offspring born and reared on lettuce. This was the highest percentage of infection obtained in any of the experiments with the lettuce-fed offspring of virus-bearing aphids. In the field the aphids from the same lot produced 2 infections from 10 inoculations. These from the second generation gave 30 per cent of infection in the field and 8 per cent in the insectary experiments. The third generation produced 8 per cent of infection in the insectary and 19 per cent in the field, while the fourth generation produced 4 per cent of infection in the insectary and none in the field. There was a gradual increase in the length of the average incubation period of the disease, from 18.1 days with the original virus-bearing aphids to 37 days with the fourth-generation offspring.

The results of the transfers of the Norfolk strain of *Rhopalosiphum persicae* were similar to those of local *Macrosiphum solanifolii*. With *R. persicae*, however, smaller percentages of infection were obtained

in nearly every case. The fourth generation produced no infection in the insectary experiments, although in the field one infection was obtained. The incubation periods of the disease in every instance were shorter than in the *M. solanifolii* series. Both lots of plants were kept under similar conditions and treated alike, and the infectious entity which the insects carried was derived from the same source. Observations of the feeding habits of the two species reveal that individuals of *R. persicae* were less disturbed by transference and not as liable to leave the plants as *M. solanifolii*. The former also fed considerably more during the 48-hour period of inoculation than *M. solanifolii*. It is possible that these slight differences in the feeding habits of the species would result in the transmittal by *R. persicae* of the more active infection, thereby shortening the period of incubation.

Macrosiphum solanifolii (Alabama strain) produced infection with all but the second generation under insectary conditions and the first and fourth generations on the field plants. The reason for not obtaining infection with the first and second generations in the instances cited may be due to several causes. These can not be explained until more is learned concerning the relationships existing between the insects and the causal factor of spinach-blight. The incubation periods for this series correspond closely to those obtained with the Norfolk strain of *M. solanifolii*. The inoculations with *R. persicae* (Louisiana strain) produced about the same number of infections as did *M. solanifolii* (Alabama strain) with the exceptions that no infections were obtained with the second generation under insectary conditions or with the fourth generation either in the field or in the insectary. The incubation periods of the disease were similar with both species.

It has been shown that certain supposedly non-virus-bearing aphids, collected locally on plants other than spinach and their offspring for several generations may occasionally produce infections of blight when allowed to feed on healthy spinach. No infections were obtained in any case where the aphids from other States were placed on healthy spinach plants, unless they had previously fed on diseased plants. The offspring of virus-bearing aphids when transferred to healthy spinach in previous experiments have occasionally caused infections of blight. This ability on the part of the aphids is confirmed by the results obtained in this experiment. The preceding data indicate that the inciting factor of spinach-blight is transmissible by parent aphids to their offspring for several generations. How this is accomplished is not known and probably will not be fully understood until the nature of the inciting factor of this disease has been discovered. There are undoubtedly several factors which exert a controlling influence on the ability of aphids to transmit the disease, when the causal factor is inherited from ancestors which have fed on blighted plants. From the data collected on the conditions which limit this factor the following appear to be the more important: The con-

dition of the diseased plant at the time it was used as food by the original ancestors; the temperature and humidity which prevailed both during the period when the aphids were feeding on the diseased plant and when their descendants fed on the healthy plants; and the parts of the plants on which the aphids fed. It is of importance to note in this connection that many aphids, although they may have fed on a diseased plant, are not necessarily virus carriers. From the data collected in the greenhouse and insectary experiments it appears that not over 50 or 60 per cent of the virus-bearing aphids produced blight in the healthy plants to which they were transferred. Likewise, many plants show a temporary immunity to the disease. In several of the earlier series plants were inoculated with a known virus and remained healthy for 72 days, at which time they were reinoculated. Infections occurred, and positive symptoms of blight appeared 17 days later.

Since it is possible under certain conditions for aphids to transmit the causal factor of spinach-blight to their offspring, thereby enabling the latter to produce infections in healthy plants on which they feed, it is of interest to note that, while it indicated a closer association between a plant pathogene and its transmitter than has hitherto been known to exist, yet there occur among animal and human diseases several instances where the relationships between the definitive parasites and their transmitters are similar to those found with spinach-blight.

For many years it has been known that certain animal diseases are communicable by the transference of virus from diseased to healthy individuals. The viruses are usually highly infectious and, although filterable, are thought to contain elements of a parasitic nature which thrive and reproduce in one or more hosts. Many zoologists have considered that these ultramicroscopic, infectious entities are probably protozoans. Several of the more important human diseases belong to the group caused by filterable virus. Little is known concerning their nature, except in some cases the method of their transmission, their alternate hosts, and various points in their life history. Knowledge of the definitive organism is lacking in each case.

Recently it has been found that there is a group of plant diseases that are caused by filterable virus. Studies of certain of these diseases indicate them to be of parasitic origin, and from the nature of spinach-blight—namely, that it is caused by an infectious virus, and that no microscopic organism has been found associated with the disease—it constitutes another addition to this peculiar group of plant diseases.[1]

[1] Through the courtesy of Dr. L. A. Hawkins, of the Bureau of Plant Industry, the juice of diseased spinach plants which had been filtered through a Berkefeld filter and a porous clay cup was obtained for inoculation purposes. A limited number of inoculations were made, but unfavorable cultural conditions caused the inoculated plants to yield rather uncertain results; therefore it seems advisable to repeat these experiments under more favorable conditions before the results are published.

There are, then, two groups of diseases: One affecting animal life, the other affecting plants. The pathogens in the groups have several points of similarity. First, they are evidently parasitic; second, they are caused by an infectious virus; third, their manner of transmission from diseased to healthy hosts is usually through the agency of animal parasites infesting the hosts—that is, insects, mites, or ticks; fourth, students of both groups of diseases generally believe the definitive parasites to be ultramicroscopic organisms. Among animal diseases there are several which are transmitted by insects, mites, or ticks. Rocky Mountain spotted fever, a disease caused by an infectious virus is carried from diseased to healthy hosts by several species of ticks. Ricketts [1] found that not only was infection carried by the adult tick but a percentage of the offspring of the ticks from diseased animals inherited the ability to produce the disease. This case is similar to spinach-blight in which the causal factor of the disease is transmitted by the parent aphids to their offspring. The infectious entity of yellow fever has not been definitely proved to pass from adult mosquitoes to their offspring, yet the later experiments by Finlay [2] indicated the probability that the infectious entity was hereditary in certain species of Calopus.[3]

The well-known Texas fever of cattle was found by Smith and Kilbourne to be caused by a definite organism. The organism is transmitted by ticks on affected cattle to their offspring.

SUMMERING OF SPINACH-BLIGHT

ALTERNATE HOSTS

If the disease survives the summer on plants other than spinach, it is probably carried by aphids both to and from the other species of plants. Certain insects feed on spinach only in the spring and fall; hence, it is possible that it may be carried by one of these species instead of by those commonly inhabiting the spinach during the winter as well as in the spring and fall. The combined known food plant list of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* comprises more than 100 species, representing a wide range of botanical groups, any of which might possibly be alternate host plants of the disease. So far as time would permit, those species closely allied botanically to spinach have been carried through series of inoculations the results of which were negative. Many species appeared to be affected with mosaic diseases, and these were used to inoculate healthy spinach plants, but without success in producing infectious blight. Only by a great amount of systematized work entailing thousands of inoculations can any definite proof be obtained relative to the question of alternate hosts of the inciting factor of spinach-

[1] Ricketts, H. T. Spotted fever report. no. 1/2. *In* 4th Bien. Rpt. State Bd. Health Mont. 1907–8, p. 87–191. 1908.

[2] Finlay, C. J. Trabajos selectos (selected papers). 657 p. Habana, 1912.

[3] Smith, Theobald, and Kilborne, F. L. Investigations into the nature, causation, and prevention of southern cattle fever. *In* U. S. Dept. Agr. Bur. Animal Indus. 8th/9th Ann. Rpt., 1891/92, p. 177–304, 20 pl. 1893.

blight. Thus far it has been impossible to give this phase of the problem more than the briefest consideration. Further data along this line are being accumulated as the experiments proceed.

RELATIONSHIP OF INSECTS TO THE SUMMERING OF THE DISEASE

As certain aphids have been found to possess the ability to transmit the causal factor of spinach-blight to their offspring, the question arises, Do aphids then serve as a means of carrying the disease over from the time the crop is harvested in the spring until the spinach is planted in the autumn? The evidence we have would indicate that succeeding generations from the original parent aphid from diseased spinach gradually become less infective and the percentage of virus-bearing offspring decreases with each generation, provided they do not have access to diseased food. The condition of the diseased plant at the time the parent aphids feed on it is an important determining factor in the transmission of blight by their offspring. Also, the numbers of the aphids are reduced to a minimum in July and August, which would mean that a very small percentage of those which have the earlier conditions entirely favorable would survive to produce offspring which eventually reach the spinach in the fall. When blight first appears in the autumn, it occurs usually as widely separated cases, from which infection is carried to surrounding plants by aphids. In fact, the first blighted plants to be found in the autumn are about as numerous as the aphids bearing virus by heredity might be expected to be. In the autumn of 1917, collections were made of numbers of both *Macrosiphum solanifolii* and *Rhopalosiphum persicae* from various cultivated plants and weeds and placed on healthy spinach seedlings for a few days. Positive results were obtained with 10 adults of *M. solanifolii* collected on the vines of sweet potatoes (*Ipomoea batatas*) on September 24, 1917, and with three adults of *R. persicae* collected from celery (*Apium graveolens*) on October 1. A number of *M. solanifolii* were placed on five spinach seedlings in the insectary, and two of them developed symptoms of blight on October 30. Similarly, on November 1, positive symptoms appeared on one of the two plants on which the individuals of *R. persicae* were allowed to feed. At the time the insects were collected, healthy spinach plants were inoculated with the juice of the plants from which the aphids had been collected. These inoculations gave negative results.

On June 4, 1917, in the potato field on farm A, which had been in spinach during the winter and early spring, a number of *Macrosiphum solanifolii* were collected on wild mustard plants growing near the center of the field where blight had been serious during the previous winter. These aphids were placed on pots of healthy spinach plants in the greenhouse; similar pots of plants were kept as controls. Seven days later some of the spinach plants showed doubtful symptoms of blight. Six days after this some of the plants developed the characteris-

tic symptoms of blight. These results indicate that *M. solanifolii* on the wild mustard plants were still virus bearers, although they or their parents had not fed on spinach for some time, as the spinach in this field had been cut early in March. Inoculations with juice of the mustard plants gave negative results.

INFECTIOUS ENTITY OF SPINACH-BLIGHT MAY BE CARRIED FROM SPRING TO FALL BY A DIRECT LINE OF APHIDS

On April 6, 1917, a blighted plant was obtained from farm B and brought to the greenhouse to use for inoculations. One adult of *Macrosiphum solanifolii* was removed from the blighted plant and placed on the larger of two spinach plants 74 days old, growing in a pot. A similar pot of plants served as a control. Both of the plants in the pot to which the aphid was transferred developed positive symptoms of blight, while the controls remained healthy.

On May 7, 1917, both of the inoculated plants were in advanced stages of blight. The direct descendants of the adult aphid, used for the inoculation on April 6, were abundant on these blighted plants. On May 7 some of these direct descendants were transferred from the blighted spinach plants to a small potted eggplant which had been grown in a large cage under insect-free conditions. This eggplant was then removed from the pot and transplanted to the soil in a field cage which was free from spinach. Several potato tubers were planted in the soil about the eggplant. On May 14 several Ruby King pepper plants which had been grown under insect-free conditions were transplanted to the above field cage, and to them were transferred young aphids from the eggplant. These aphids were kept in a field cage and allowed to reproduce and feed only on eggplant, pepper, and potato plants during the summer.

On August 9, 1917, a number of the above aphids in various stages of development were transferred to healthy spinach seedlings growing under insect-free conditions in another field cage. Similar seedlings in another field cage served as controls. On September 1 some of the plants to which the aphids had been transferred on August 9 were slightly mottled, while the control plants were of a normal green color. The aphids multiplied rapidly on the spinach plants in the field cage, and by October 1 the majority of the plants had died without showing any decided mottling. On October 6 new spinach seed grown in New York State was broadcasted in this cage and raked into the soil. A thick stand of spinach seedlings came up in six days. To these seedlings the aphids migrated from the few yellowish plants which still remained alive. On November 10 a few of the plants from the broadcasted seed had developed the mottled leaves characteristic of blight. On November 15 a considerable number of the seedling plants in this cage had yellow cotyledons, and the true leaves of many were distinctly mottled. At this time only one of the original plants growing in this cage remained

alive, and it had only a small whorl of mottled leaves at the center, the older leaves having degenerated, similar to those of blighted plants in the field. On November 15 one mottled leaf was removed from this remaining large plant, and with it a pot of several spinach seedlings growing in a greenhouse cage was inoculated by mashing the diseased tissues into the leaves of the seedling. Seedlings in a similar pot were mashed with a flamed needle to serve as controls. On November 27 some of the seedling plants in the pot inoculated on November 15 had developed the typical mottled leaves.

On November 15 two mottled spinach seedlings from the seed planted in the field cage on October 6 were removed from this cage, and with them one pot of healthy spinach seedlings growing in a greenhouse cage was inoculated by mashing the diseased tissues into the potted seedlings. A similar pot of seedlings was mashed with a flamed needle and served as a control. On November 27 mottled leaves had developed on the inoculated seedlings, but the control plants appeared healthy.

On August 9, when the aphids were transferred from the field cage in which they had summered to spinach seedlings in another field cage, some leaves from the potato and pepper plants on which they had summered were removed from the field cage, and potted spinach plants in a cage in the greenhouse were inoculated with them by mashing their tissues into the leaves of the spinach seedlings. These spinach seedlings were under observation until November 27, but no signs of mottled leaves developed, and the plants grew to a large size and had the dark-green color characteristic of healthy spinach plants. These results indicate that the pepper and potato plants on which the aphids had fed during the summer did not act as alternate hosts for the spinach-blight virus.

The record of the direct line of aphids kept on pepper, eggplant, and potato plants in a field cage during the summer and then transferred to spinach at about the time early spinach is planted in the field in the fall indicated that a direct line of aphids from a known virus-bearing parent may carry the infectious entity of spinach-blight over the summer even though they do not feed on spinach during that time. This record substantiates the other evidence which has been accumulated and points toward the probability that aphids are the important factor in the summering of the spinach-blight virus.

RELATION OF LEFT-OVER SPINACH PLANTS TO THE SUMMERING OF SPINACH-BLIGHT

It often happens that, when spinach fields are plowed after the late spring crop has been harvested, plants may be left growing along the edges of the field. On July 8, 1917, several diseased plants were found which had escaped the plow earlier in the season. Either species of aphids whose life averages between 30 and 40 days could pass the time elapsing

between the 1st of July and the time the early fall spinach is above the ground by four last-born generations; hence, in this case one of the factors hindering spring-to-fall transmission of the disease by aphids would be eliminated.

At the edge of a field on a farm near the Station one spinach plant left from the spring crop was observed on June 4, 1917. This plant had been left because it was in line with a cucumber row. It did not appear to have typical symptoms of blight, although some of the leaves were yellow. A number of *Macrosiphum solanifolii* were present on this plant. One leaf-bearing aphid was removed and brought to the greenhouse, where the aphids were placed on a pot of spinach seedlings. Another pot of seedlings was inoculated by mashing the tissues from the field plant into the leaves of the healthy seedlings. Similar pots of plants were pricked with a flamed needle to serve as controls. Fourteen days later, in the pot to which the aphids had been transferred, there were several plants with the mottled leaves characteristic of blight. Twenty-six days after inoculation three plants having characteristic symptoms of blight were observed in the pot of seedlings which had been inoculated with the leaf of the field plant from which the aphids were removed. These results indicate that the few plants which are allowed to remain in the fields after the regular crop has been harvested are the collecting places for numerous aphids, thus making the possibilities great for such plants becoming diseased with spinach-blight, and serving to carry the disease well into the summer after the regular crop has been harvested. It is known that this field plant grew for some time after aphids had been transferred from it to the greenhouse. A rank growth of weeds eventually surrounded this plant, so that it was impossible to determine how long it remained alive and served as a source of infection. The evidence which we have points toward the probability that aphids are instrumental in carrying the disease over the summer by their power to transmit the casual factor from parent to offspring for several generations. Probably this is not the only means by which spinach-blight may pass the summer period. As more is learned concerning the relationship between blight, insects, and plants other than spinach, doubtless other means may be discovered.

ABILITY OF OTHER INSECTS INFESTING SPINACH TO TRANSMIT SPINACH-BLIGHT

With the exception of the bean aphis, none of the species included are generally active during the winter months, and for these reasons are not liable to become important, from their agency as transmitters of blight virus. Experiments were performed in which most of the insects infesting spinach in this region were transferred from diseased to healthy plants. The insects remained on the plants in each case for 48 hours.

BEAN APHIS (*Aphis rumicis* Linnaeus).—Should this aphis become abundant at a time when blight is prevalent, it will undoubtedly be an important factor in the transmission of the same, but the species is generally abundant only during the summer months and is rarely found on spinach in this region. On Long Island, New York, it occurs on spinach grown for seed. Specimens received on July 12, 1917, on blighted spinach plants from Long Island were placed on healthy spinach seedlings. Infections occurred, and positive symptoms of blight appeared on August 2, thus proving the ability of the species to transmit the disease, as well as giving a record of the occurrence of blight on Long Island.

TARNISHED PLANT BUG (*Lygus pratenis* Linnaeus).—Two infections were obtained when specimens from blighted plants were transferred to 10 healthy spinach seedlings. This species occurs abundantly on spinach growing after March 20. As late-grown spinach is usually not harvested until April it is possible that this insect may be partially responsible for outbreaks of the disease at this time. In the autumn occasional specimens of *L. pratensis* have been collected on spinach as late as November 25, but our records would not indicate that they are sufficiently abundant to be of importance in causing early infections of blight.

SOUTHERN CORN ROOTWORM (ADULT) (*Diabrotica 12-punctata* Olivier).—This insect occasionally feeds on spinach when other preferable food is scarce. No infections were obtained when individuals were transferred from diseased to healthy spinach plants.

GREEN PLANT BUG (*Nezara hilaris* Say).—This species has been collected on spinach in October and in April. Individuals which were known to have fed on a diseased plant were transferred to healthy plants and allowed to feed on them for 48 hours. No infections resulted from these transfers. A few individuals of *Euchistus servus* Say have been collected on spinach, and some from a diseased plant were transferred to known healthy plants and allowed to feed. They gave negative results so far as obtaining a transmission of blight was concerned.

Thrips tabaci Lindeman, *Smynthurus hortensis* Fitch, and *S. quadrimaculatus* Ryder may occur abundantly on spinach during the fall and spring, but no infections were obtained when these insects were transferred from diseased to healthy plants.

OCCURRENCE OF SPINACH-BLIGHT IN OTHER STATES

NEW YORK.—On July 14, 1917, diseased spinach plants from western New York were received. These showed the typical characteristics of blight. Virus was obtained and inoculated into 15 healthy spinach seedlings in a field cage. Ten healthy spinach seedlings were used as controls. On July 25 nine of the inoculated plants had developed symptoms of blight, and by August 1 eight were distinctly mottled, and the leaves malformed. Seven of the inoculated plants died shortly after

the inoculations had been made. No evidence of the disease appeared on any of the control plants. On August 6 virus was obtained from the blighted plants and used to inoculate several seedlings grown in a pot in the greenhouse under insect-free conditions. A similar pot of spinach seedlings was untreated and served as a control. On August 22 it was observed that the plants inoculated on August 6 had developed the characteristic mottled appearance of blight. All the control plants remained healthy. This gives definite proof that spinach-blight occurs in western New York as well as on Long Island.

OHIO.—Two boxes of diseased spinach plants of the Viroflay type were received from Ohio. These plants were in various stages of what appeared to be typical spinach-blight. In Ohio the disease is known by the name of "yellows" (Pl. 10, A).

Individuals of both *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were present on the plants. Aphids were removed and placed on pots of Savoy spinach seedlings growing in the greenhouse. Similar plants were used as controls. Mottled leaves were removed from each of the plants from which the aphids were taken, and Savoy spinach seedlings were inoculated by mashing the mottled leaves into them. Ten days after inoculation it was observed that some of the potted seedlings were showing doubtful symptoms of blight. Six days later some of the inoculated plants had developed characteristic symptoms. The control plants remained healthy.

At the same time the inoculations were made in the greenhouse, plants in one of the field cages were inoculated by mashing the diseased tissues from the Ohio spinach into the leaves. A number of plants were thus inoculated, and a similar number were pricked with a flamed needle serving as controls. Sixteen days after inoculation some of the plants in the field cage had developed typical symptoms of blight, but the control plants remained healthy. Six days later one of the mottled leaves was removed from each of the two blighted plants in the field cage. Pots of spinach seedlings 16 days old were inoculated by mashing the diseased tissues into the cotyledons of the seedlings. A similar pot of plants served as a control. Eight days after inoculation 14 of the plants developed positive symptoms of blight, the control plants remaining healthy. Twelve days after observing positive symptoms of blight in the field cage one mottled leaf was removed from a blighted plant, and with it 16 plants 20 days old and 29 plants 11 days old were inoculated by mashing the diseased tissues into the cotyledon and the first true leaves. Eleven days after inoculation the majority of the plants in each of the two pots had developed mottled leaves, but the control plants remained healthy.

From these results it appears that the Ohio spinach is subject to the same disease which is present in the Norfolk section and that the disease is caused by the same virus which causes spinach-blight in Virginia. It is interesting to note that spinach-blight virus is evidently readily trans-

ferable from the Viroflay to the Savoy type, and vice versa, thus indicating that the same disease may develop in various sections where spinach is grown, regardless of the type of plants used.

SOIL TRANSMISSION OF SPINACH-BLIGHT

To determine whether spinach-blight is carried in the soil, blighted areas were selected in each of three fields on an adjoining farm. The blighted plants were removed from the areas in each field, and one flat was filled with soil from each area, care being exercised to take the soil from around and under where the blighted plants had developed. The three flats of soil were brought to the greenhouse and covered with cloth cages. A fourth flat of the same size was filled with steamed greenhouse soil to serve as a control. The following night the greenhouse was fumigated with tobacco to kill insects. Three days after bringing the flats to the greenhouse they were planted with four lots of spinach seed from different sources. The control flat was planted with similar seed. Previous to planting, the seed was soaked in 1 to 100 formaldehyde for a few minutes. The four lots of seed in each flat were separated from one another by thin board partitions. For a period of 75 days these flats were left in the greenhouse. No signs of blight developed in any of the plants. The plants in the flats of field soil were as healthy as those from the same seed grown in steamed soil. Therefore it would appear that spinach-blight is not carried in the soil.

To insure that neither the transfer of soil from the field to the greenhouse nor the modified conditions of the greenhouse was responsible for the failure of blight to develop on the plants grown in the various lots of soil, this experiment in somewhat different form was duplicated in the field on an adjoining farm. A bed of spinach which had a number of blighted areas in it was used. Four areas were selected where uniform outbreaks of blight had developed. The blighted plants, including the roots, were removed from the soil, and all traces of vegetation which might harbor insects were removed from the first three areas selected. In the fourth area the blighted plants were left exactly as they grew in the field. A trench the size of a cage approximately 2 by 3 feet in area was dug, the cage set therein, and the soil well banked about the outside in order to insure that no insects should enter from below. After the four cages had been placed, the soil within them was treated as follows:

In cage 1 the soil was loosened and planted to two lots of spinach seed which had been disinfected in 1 to 100 formaldehyde for a few minutes before planting. Four days later, before the spinach plants were up, the soil in this cage was soaked with a 40 per cent nicotine-sulphate solution (1 to 100) in order to kill any insects. Cage 2 was planted with spinach seed which had been disinfected with 1 to 100 formaldehyde. The soil in this cage was left in the same condition as after it had grown blighted plants, no attempt being made to free it of insects. In cage 3 the soil was treated in

a manner similar to that in cage 1 and was planted with two different lots of spinach seed. In cage 4 seven of the original field plants were present. Of this number one was apparently healthy while the others were in various stages of blight. Care was exercised not to disturb the aphids which were feeding on the plants. Four commercial lots of spinach seed were planted in this cage, care being taken to include lots planted in each of the other cages. No attempt was made to free this cage of aphids or other insects by the use of nicotine solution. Twelve days after planting it was observed that spinach seedlings in each cage were growing vigorously. Twenty-four days after planting, the young plants in all four cages appeared healthy, but aphids were observed on the seedlings immediately surrounding the mature plants in cage 4, while none were present in the three other cages. Ten days later the seedlings in cages 1, 2, and 3 all appeared healthy, while numerous seedlings in cage 4 bore wrinkled, much mottled leaves characteristic of blight. Four days later seedlings in cage 4 were dwarfed and mottled, and the cotyledon leaves were yellow and were dying. Many aphids were observed on the seedlings in cage 4, but none were seen on any of the seedlings in cages 1, 2, and 3. Seven days later all but a very few of the plants in cage 4 were dwarfed and mottled, while the plants in cages 1, 2, and 3 appeared healthy. Nine days later photographs were taken to show the characteristic blighted appearance in cage 4 on that date (Pl. 10, B) as compared with the healthy plants in cage 2 (Pl. 11, A). Healthy greenhouse plants were inoculated with the tissues of diseased seedlings from cage 4. These eventually developed typical symptoms of blight. Similar plants used as controls remained healthy. The plants in all four cages continued to grow until late in May, when the aphids became so abundant in cage 4 that practically all of the plants were killed. During the 44-day period after planting the seed the plants in cages 1, 2, and 3 grew more rapidly and became much larger than those in cage 4, showing no symptoms of blight. About six weeks after planting, the cloth door of cage 3 was found open, probably owing to a severe wind storm the night before. About 500 plants were removed from cage 3 at this time in an attempt to find aphids or blighted plants. Neither were found; so the remaining 500 plants or more were left in the cage. The experiments in cages 1 and 2 continued until the end of the season without the plants in either cage showing signs of blight. Some time after the door of cage 3 was found open, aphids were observed on the plants within this cage, and before the end of the season spinach-blight had developed on some of the plants. The results obtained in these four cages substantiate those obtained in the greenhouse and indicate that spinach-blight is not carried in the soil, but is carried from plant to plant by insects or mechanical means.

SEED TRANSMISSION OF SPINACH-BLIGHT

The occurrence of spinach-blight from time to time in commercial fields, and especially in new land which had never been planted to spinach before, suggested the possibility of seed transmission of this disease. During the period that the various experiments on the transmission of this disease have been conducted many thousands of spinach plants have been grown, some even to maturity in both greenhouse and field cages. In no case has there been any evidence that blight was transmitted through seed. When it is considered that the various seed strains used for this work have been obtained from practically all parts of the United States and Europe where spinach seed is grown, it would appear that the weight of data is against the idea of seed transmission of spinach-blight.

During the spring of 1917 seed was collected from over a hundred blighted plants in various stages of the disease. Unfortunate circumstances following the harvesting of this seed made it impossible to get immediately as many data as were desired relative to the transmission of spinach-blight by these seed; therefore this phase of the problem is not complete. The growing of spinach plants from the seed of these diseased plants is being conducted on a rather extensive scale, both in the field and in the greenhouse.

POSSIBLE MEASURES OF CONTROL

CONTROL OF INSECT CARRIERS OF THE INFECTIOUS ENTITY

Probably the most effective and immediate control of spinach-blight can be obtained by destroying or otherwise eliminating the transmitters of the infectious entity of the disease. As aphids are usually the most abundant insects on spinach in this region during the time blight is present, efforts are being made at the present time to control them. During the winter parasites of the aphids are not as effective in holding them in check as they are during warmer periods. Also the predacious enemies of the aphids, particularly the ladybird beetles *Hippodamia convergens* Guérin and *Megilla maculata* De Geer, are hibernating and are of little benefit in reducing outbreaks of aphids between November 1 and April 1; hence, except for temperature conditions, natural control of aphids can not be counted upon to relieve the situation at this time. Experiments have been conducted for several years on spraying spinach for the control of aphids. It was noticed in 1914 and again in 1916 that on sprayed plots where the most effective control of aphids was obtained there was much less blight than on the unsprayed plots where the aphids were allowed to feed undisturbed. Not until the autumn of 1917 was an arrangement devised whereby spinach plants could be effectively sprayed to kill the plant lice. The present indications are that blight can be materially reduced by the timely application of sprays for the control of aphids.

Extensive work along this line is now under way, and it is hoped that by another season the results will have borne out their present indications and methods devised which may be practically applied on a commercial scale.

EXPERIMENTS ON PRODUCTION OF BLIGHT-RESISTANT SPINACH

In the fall of 1916 a number of strains of the Savoy spinach were obtained from various parts of the United States and Europe. These seed were planted in separate beds on a piece of land about 1 acre in area, where in 1914 spinach-blight was so serious that not a barrel of marketable spinach was harvested. Blight developed again in 1915 and killed a large percentage of the plants before spring, but a few plants remained alive and produced seed. The seed from each plant was saved separately. In 1916 it was planted on the same land. Additional strains of commercial seed were again planted on this land, as was also the supply of seed from crosses of various types of spinach, including some importations from Asia, grown at Concord, Mass., by Mr. J. B. Norton, of the Bureau of Plant Industry. It was observed that the 1915 selections from commercial strains were superior both in type of plants and in disease resistance to the commercial strains used in 1916. It was also observed that the seed furnished by Mr. Norton showed greater resistance to blight than any of the other lots of seed (Pl. 11, A). Plants from the Massachusetts-grown seed varied widely in types, but a limited number of good Savoy plants were present. In the spring of 1917 seed was saved from the Savoy plants of the Massachusetts-grown strain, from the 1915 selections and from the commercial strains. During the fall of 1917 the various lots of selected seed were tested further, both on the experiment station farm and to a limited extent on a number of widely separated commercial fields. Although the results obtained thus far are encouraging, the nature of spinach-blight and its method of dissemination is such that it seems best to consider that the above experiments point to a possible means of control, rather than an immediate solution for the problem. Breeding experiments are being continued with the Savoy spinach and also with several types of spinach which are used commercially in other parts of the United States.

SUMMARY

(1) Spinach-blight has caused a greater annual loss to the trucking interests of eastern Virginia than any other single disease. It has been conservatively estimated that spinach-blight causes an annual loss of at least $200,000.

(2) Spinach-blight is a specific disease characterized by a mottling and malformation of the leaves and a decided stunting of growth. The diseased plants go through a number of characteristic stages and finally die. Diseased plants may occur in definite areas or they may be scattered over the field.

(3) Spinach-blight is distinguished from fungus diseases by the fact that there is no specific microscopic organism associated, and that the various fungi produce definite leafspots, while spinach-blight causes a gradual degeneration of the tissues.

(4) Opinions vary as to the time when spinach-blight first appeared in eastern Virginia. One grower reports it as serious at least 13 years ago.

(5) Mr. L. L. Harter, of the United States Department of Agriculture, worked on malnutrition diseases of various truck crops, and it is evident from his published reports that spinach-blight was included among these diseases. The early work on these diseases resulted in the use of better cultural methods.

(6) This blight has increased in seriousness from year to year. During the past 10 years the disease has spread until it is now present annually throughout eastern Virginia wherever spinach is grown commercially.

(7) For some years past it has been observed that spinach-blight became most serious within a short time after aphids were observed to be abundant on the plants. These observations led growers to suspect that the aphids were the direct cause of this disease.

(8) Some spinach growers were of the opinion that spinach-blight was due to poor soil drainage. Data relative to this point proved that the drainage within blighted areas was usually as good and often better than that in other parts of the field where the plants were healthy.

(9) Fertilizer experiments conducted in 1915 and 1916 proved that stable manure, lime, and commercial fertilizers applied to land where spinach-blight had been very serious the year previous had no direct influence on the development of the disease. Experiments with the substitution of fertilizer elements not commonly used, for those generally employed in making up commercial fertilizers had no effect in reducing the amount of blight.

(10) By a repetition of earlier work in an attempt to determine whether or not fungi or bacteria were associated with blighted plants, it was found that tissues of plants in early stages of the disease remained sterile on plates of nutrient media. Plants in more advanced stages of spinach-blight, where the tissues were breaking down, yielded numerous fungi and bacteria. When healthy spinach plants were inoculated with pure cultures of each of these organisms, no blight was produced.

(11) Inoculations made in the winter of 1915–16 with the juice of blighted spinach plants gave indications that the disease was of an infectious nature.

(12) In eastern Virginia spinach is grown in the fall, winter, and early spring. During this period two species of Aphididae, the potato aphis (*Macrosiphum solanifolii* Ashmead) and the spinach aphis (*Rhopalosiphum persicae* Sulzer) are the most abundant insects on spinach. Several other species of insects are found associated with spinach during

early fall and late spring, but thus far experiments have proved these of minor importance in connection with spinach-blight.

(13) Differences in the habits of the aphids, however, cause *Macrosiphum solanifolii* to be the more important agent in the dissemination of the disease. Both aphids infest many species of plants. Experiments to determine whether any of these may constitute alternate hosts for the inciting factor of spinach-blight are not completed.

(14) Direct transfers of virus-bearing aphids to healthy plants produced infections of spinach-blight.

(15) Inoculation with the juice of crushed virus-bearing aphids produced infections of blight.

(16) Transfers of aphids which had not previously fed on diseased material produced infections of blight in a few cases.

(17) Inoculations with the juice of lettuce, eggplant, peppers, and potato, used as food for the aphids, did not produce infections.

(18) Blighted plants collected from various local farms and from blighted areas and individual diseased plants proved to be due to similar virus in all cases.

(19) Virus-bearing aphids produced infections in healthy plants when allowed to feed on them for two minutes.

(20) The infectivity is greater with adult aphids than with those which are immature, and the incubation period of the disease produced by the adults is materially less than when the disease is produced by the immature forms.

(21) Aphids have the ability to carry infection to several healthy plants after leaving the diseased host.

(22) Supposedly non-virus-bearing aphids were found to cause infections of blight when transferred to healthy spinach. Aphids from Louisiana, Alabama, Florida, and Colorado when transferred to healthy spinach did not cause infections of spinach-blight unless they had previously been allowed to feed on diseased spinach. Inoculations with the juice of crushed aphids from other States yielded similar results.

(23) Non-virus-bearing aphids must remain on diseased plants for 5 minutes to 14 hours to become carriers of infection.

(24) Virus-bearing aphids do not lose their ability to transmit the causal entity of spinach-blight during the process of molting.

(25) Infections were obtained with the offspring of virus-bearing aphids which had not previously partaken of food.

(26) The infectious entity of spinach-blight was found to be transmitted by virus-bearing adult aphids to their offspring. It was also found that although aphids were reared on lettuce for four consecutive generations, yet a few of the fourth generation were virus bearers and produced infections when they were transferred to healthy spinach. These results show similarity to certain animal diseases caused by virus and transmitted by insects or ticks.

(27) Since it has been found that the causal factor of the disease may be hereditary with the aphids, this pointed to the possibility of its summering by this method. Experiments have shown that aphids collected on spinach plants left after the crop had been harvested may be virus bearers, as are also aphids collected from weeds growing later in the season in the same fields.

(28) Experiments with aphids from plants other than spinach during the fall produced spinach-blight in a limited number of cases. The direct offspring of a known virus-bearing aphid reared during the summer in a field cage on pepper and potato plants produced blight when they were transferred to spinach seedlings in August, or about the time early spinach is coming through the ground. Infections were obtained in a small number of cases with several other species of Hemiptera. These are probably not important as blight transmitters, as they do not occur abundantly at the time blight is prevalent.

(29) Spinach-blight has been found on Long Island and in western New York both on seed spinach and on scattering plants left from the canning crop.

(30) Blight was also found in Ohio on spinach grown by market gardeners.

(31) Experiments to date indicate that spinach-blight is not transmitted through the soil.

(32) From the data collected it is probable that spinach-blight is not transmitted by seed.

(33) The control of the aphids infesting spinach offers the most immediate possibilities for the control of spinach-blight.

(34) Experiments are under way for the breeding of blight-resistant seed, but these do not offer any immediate solution for the spinach-blight problem.

PLATE A

1.—A typical blighted Savoy spinach plant in the fifth stage of the disease. Note the difference in size and color of this plant and the healthy plant shown in figure 2. The plants were the same age and were drawn to the same scale. (Original.)

2.—A healthy Savoy spinach plant. Note the deep-green color, the tugidity, and larger size, which characterize the healthy plant. (Original.)

1

2

PLATE 1

A.—*a*, A spinach plant killed by blight; *b*, an apparently healthy plant; *c*, *d*, and *e*, plants in progressive stages of the disease. (Original.)

B.—A spinach field near Norfolk, Va., in which scattered blighted plants occur. As these are yellow, they show light in the illustration. Photographed in January, 1917. (Original.)


A
a
b
c
d
e
B

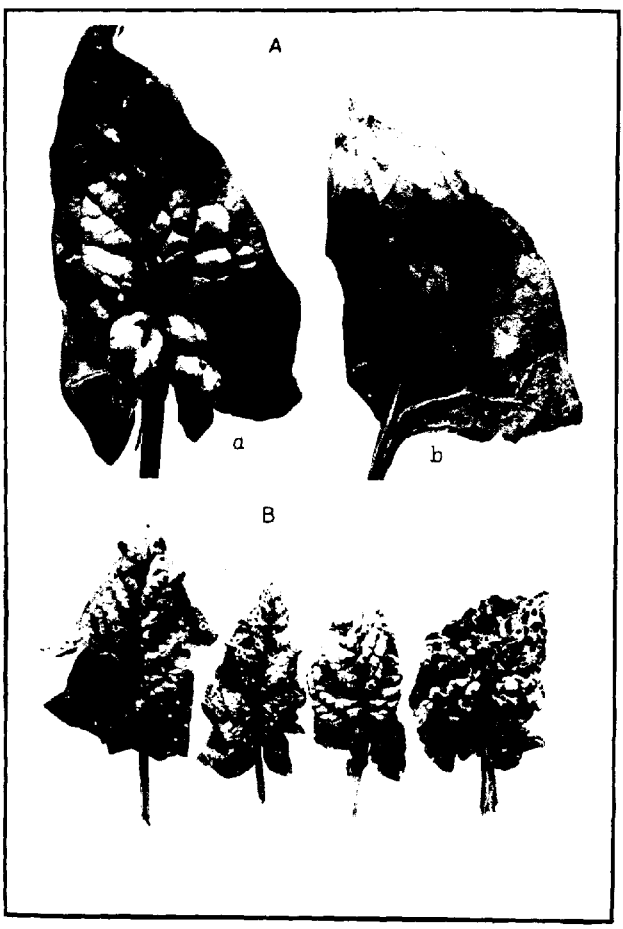
A
a
b
B

PLATE 2

A.—*a*, Upper surface of a spinach leaf affected with downy-mildew (cause: *Peronospora effusa*), showing the light areas which might be mistaken for early symptoms of spinach-blight. *b*, The under surface of a similar leaf. Note the growth of the fungus about the tip. (Original.)

B.—Spinach leaves affected with Heterosporium leafspot. This disease, besides appearing as definite black spots, may cause the leaves to become more or less yellowed, similar in appearance to blighted leaves. (Original.)

PLATE 3

A.—A spinach leaf affected with anthracnose. The degenerating tissues often have the appearance of blighted leaves, but can be distinguished by the presence of fruiting bodies of the causal fungus. Photographed by Mr. Eubanks Carsner.

B.—A spinach field showing blighted spinach plants killed by extreme cold. The healthy plants are alive and green. (Original.)


A
B


A
B

PLATE 4

A.—A spinach plant showing the first stage of the blight. (Original.)
B.—A spinach plant showing the sixth stage of the blight. (Original.)

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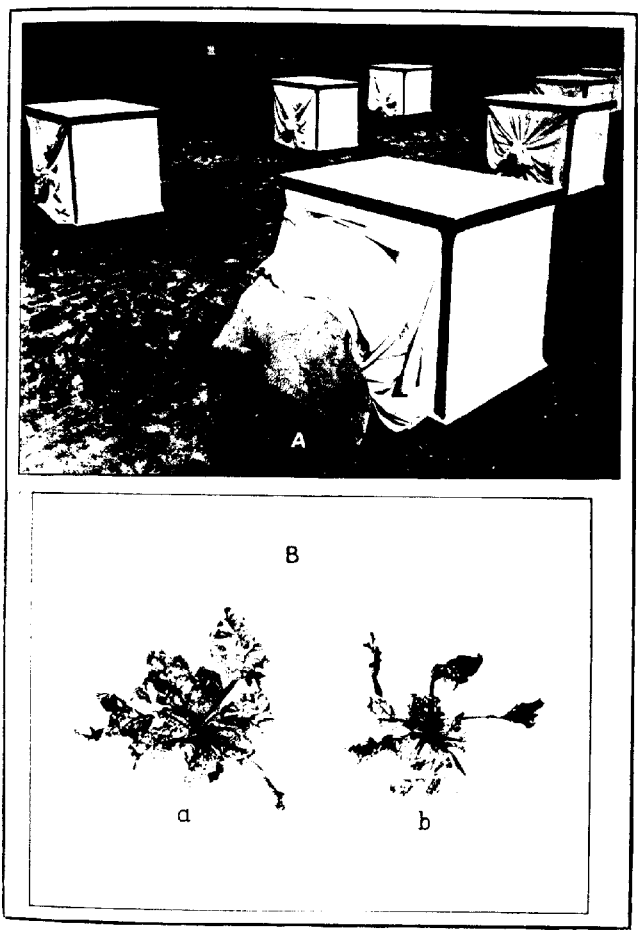
PLATE 5

A.—Improved field cage for studying spinach-blight. Note method of draping cloth around body of operator to prevent insects gaining entrance from without. (Original.)

B.—*a*, Seventh stage of spinach-blight; *b*, eighth stage of spinach-blight. (Original.)


A
B
a
b

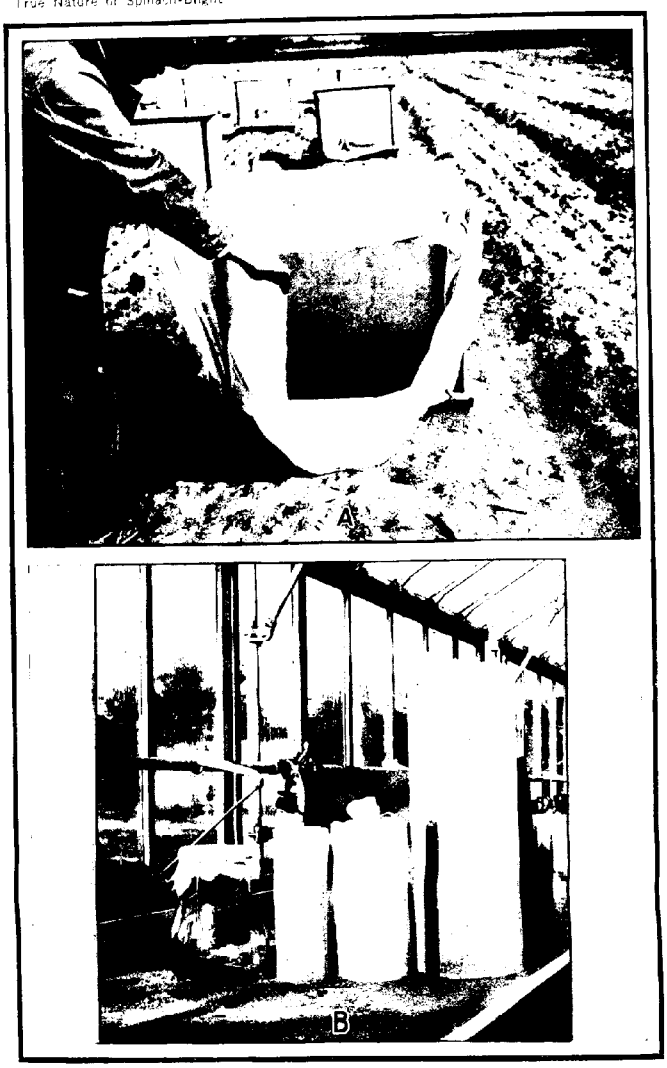

A
B

PLATE 6.

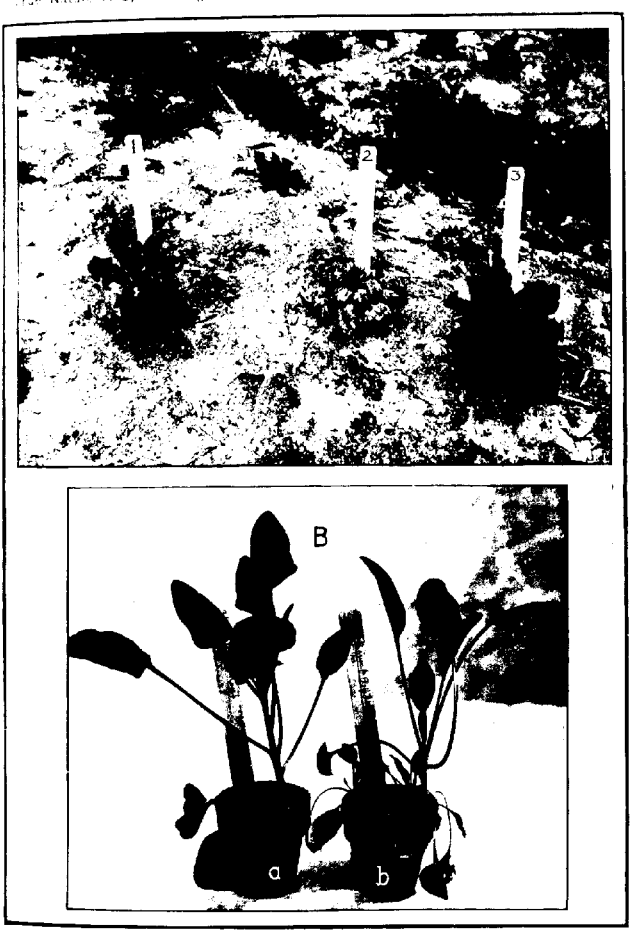
A.—Detail of the door construction of the improved field cage shown in Plate 5, A. The method of overlapping the cloth on one side, thereby avoiding the necessity of sewing the edges, should be noted. The writers are indebted to Mr. J. B. Norton, of the Bureau of Plant Industry, for suggestions relative to the construction of the door. (Original.)

B.—Cloth-covered wire cages, together with a large lantern globe, representative of the types of cage used for greenhouse experiments and for individual inoculations in the field cages. (Original.)

PLATE 7

A.—*1*, Spinach plant showing blotched appearance due to chlorosis. The juice from this plant was not infectious. *2*, Typical fifth-stage blighted plant. The juice from this plant was infectious. *3*, Healthy plant. (Original.)

B.—*a*, Healthy spinach seedling pricked with a flamed needle as a control. *b*, A pot of seedlings inoculated with the virus of spinach-blight 16 days prior to the time this photograph was taken. Note the dwarfed condition of the seedlings and the decidedly mottled leaves. (Original.)


A
1
2
3
B
a
b

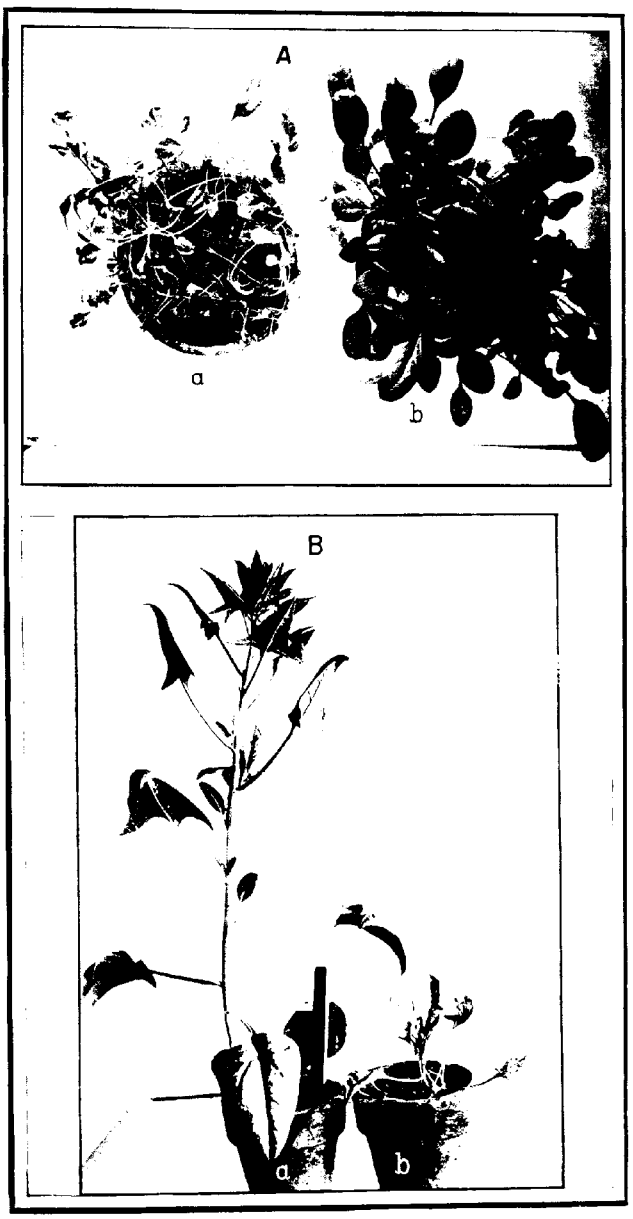

A
a
b
B
a
b

PLATE 8

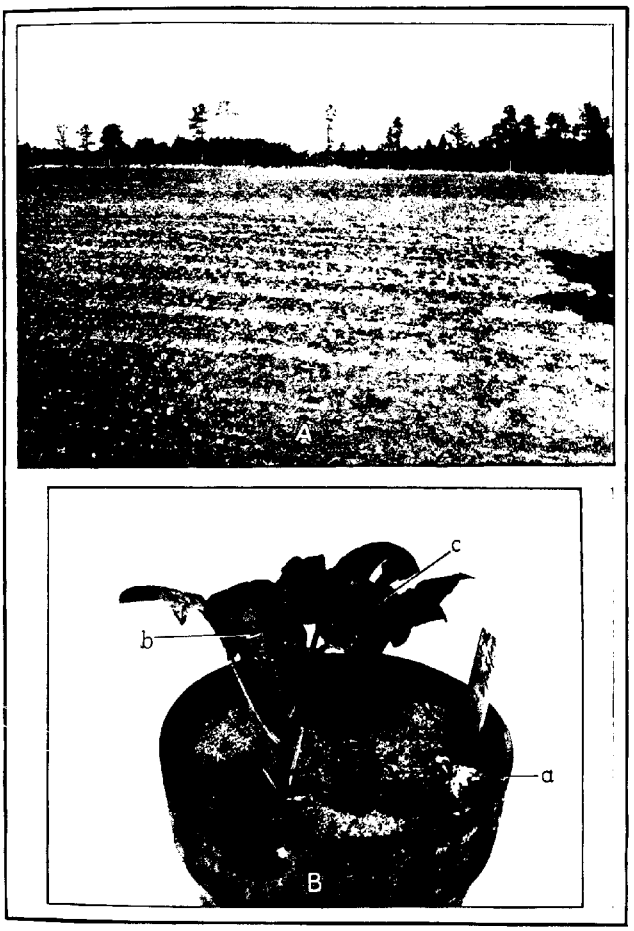
A.—*a*, A pot of spinach seedlings inoculated with virus-bearing aphids collected from blighted plants in the field. All of the plants were infected with blight. Many had died at the time the photograph was taken. *b*, A pot of healthy seedlings which served as controls. (Original.)

B.—*a*, Healthy spinach seedlings used as a control. *b*, Spinach seedlings of the same age as the control, inoculated by needle pricks with the blight virus. (Original.)

PLATE 9

A.—Large area of blighted spinach on farm E, eastern Virginia. (Original.)

B.—Three spinach plants inoculated with virus-bearing *Macrosiphum solanifolii*, first instar. Symptoms on the infected plant (*a*) developed 41 days previous to the time this photograph was taken. The two large plants (*b* and *c*) did not become infected and are healthy. (Original.)


A
c
b
a
B

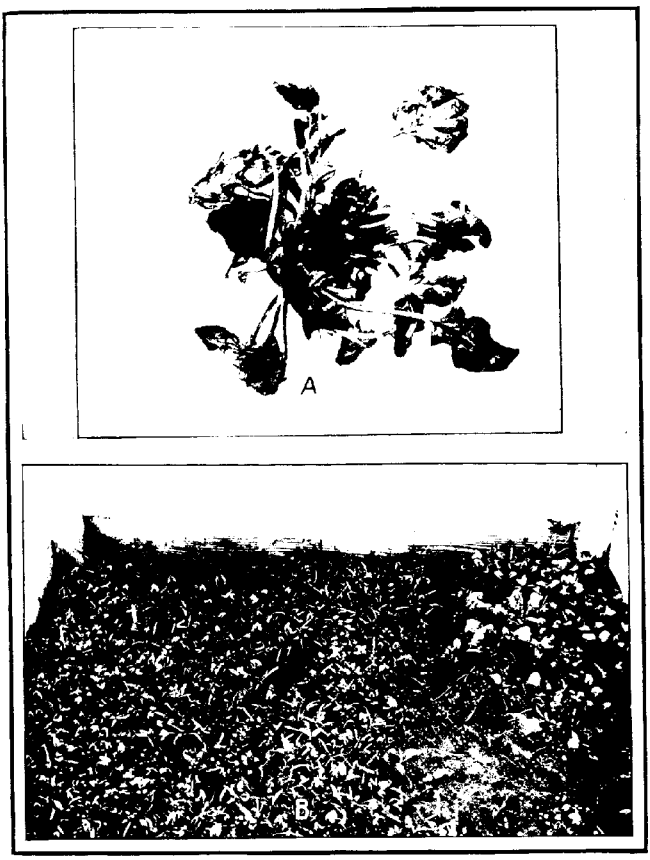

A
B

PLATE 10

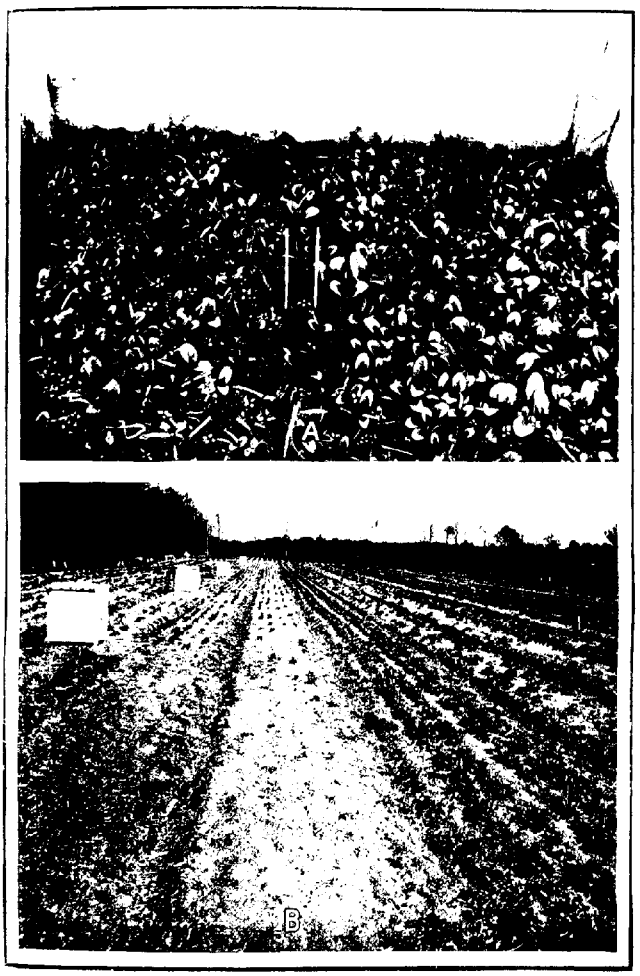
A.—A blighted Viroflay spinach plant from Ohio. (Original.)

B.—Interior of cage 4, in which the aphids and blighted spinach plants were allowed to remain. Note the stunted growth and yellowed cotyledons of the seedlings as compared with those in cage 2 (Pl. 11, A), grown under insect-free conditions. (Original.)

PLATE 11

A.—Interior of cage 2. These spinach plants are the same age as those in cage 4 (Pl. 10, B), but are healthy, as shown by their larger size and deep-green color. (Original.)

B.—Experimental plots. The bed of spinach running from lower right-hand corner of the plate was grown from seed furnished by Mr. J. B. Norton and shows a marked resistance to blight, as compared with the adjoining beds, which were planted with commercial strains of Savoy spinach. (Original.)


A
B

INFLUENCE OF GYPSUM UPON THE SOLUBILITY OF POTASH IN SOILS[1]

By PAUL R. MCMILLER,

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INTRODUCTION

The use of gypsum as a fertilizer was probably familiar to the Romans. Its beneficial effect has been noticed particularly with such field crops as clover and alfalfa, which are especially dependent upon a generous supply of potash, and its action is commonly assumed to be due to an ability to replace potassium in the soil minerals and, hence, to increase the water-soluble portion of this constituent. More recently its favorable effects upon such crops has been attributed by some investigators to its sulphur content. Some of the recently reported laboratory experiments show that applications of gypsum have a very marked effect upon the solubility of potash, while some others indicate that it either has no effect whatsoever or actually decreases the solubility of the potash.

Bradley (*3*)[2] found that gypsum added both to soils from western Oregon and to the mineral pegmatite markedly increased the content of water-soluble potash.

Dumont (*5*), studying the effect of gypsum upon both granitic soils and the separates from these obtained by mechanical analyses, found that when mixed with about one-third its weight of gypsum, moistened, and allowed to stand, the soil gave increasing amounts of water-soluble potash with lengthening periods of contact between soil and gypsum. In the case of the soil separates the fine sand showed an increase of 0.016 part per 1,000 of soil, while the coarser sands and the clay showed no increase even after 34 days' contact.

Morse and Curry (*7, p. 49–50*) found that when powdered feldspar was treated with gypsum the solubility of the potash in water was increased.

Likewise André (*2*) observed a greatly increased solubility of the potash of microcline when this was treated with gypsum.

On the other hand, Fraps (*6*), from an extended laboratory and greenhouse study of the effects of additions of gypsum upon the availability of soil potash, concludes that gypsum is often injurious. He states (*p. 30*):

> Additions of sulphate of lime . . . have no such effect upon rendering potash available to plants as has been claimed. . . .

Most recently of all, Briggs and Breazeale (*4, p. 28*) found that—

> gypsum solutions depressed the solubility of the potassium in orthoclase, the quantity of potash in solution decreasing progressively as the concentration of the calcium sulphate increased.

[1] Published, with the approval of the Director, as Paper 115 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 65–66.

Using virgin soils from Riverside, California, they found (*p. 28*) that the solubility of the potash was not—

measurably different in distilled water and in solutions of calcium hydrate or calcium sulphate.

Also in a cultivated soil from the same locality they found that the addition of gypsum actually decreased the solubility of the potash.

Apparently in none of the previously reported experiments have the conditions of contact of the gypsum with the soil been similar to those which prevail in the field. In the experiments reported below, the soils, after having the gypsum added, were allowed to remain several months in a condition of moistness similar to that found under field conditions, which, in the case of fine-textured soils in humid regions when evaporation is low and plants are absent, appears to be somewhat below the moisture equivalent (*1, p. 65*).

EXPERIMENTAL WORK

In conducting these experiments the object was to determine whether gypsum, when intimately mixed with soil and kept for some months under conditions of moistness similar to those prevailing in the field, would exert any distinct effect upon the solubility of the potash. For the experiment five soils (Table I), four from different parts of southern Minnesota and one from the Minnesota Experiment Station farm at St. Paul were employed. Sample A from near Wells is a fine-textured soil that would be classified as Fargo clay loam, according to the system of the Bureau of Soils of the United States Department of Agriculture. It is representative of a large area of poorly drained soils of lacustrine origin developed on the late Wisconsin glaciation, being highly calcareous and heavily charged with organic matter. For a soil of this texture it is surprisingly low in total potash.

TABLE I.—*Composition and physical properties of Minnesota soils used in the experiment*

Soil.	Location.	Description of soils.	Reaction.	Moisture equivalent.	Organic matter.[a]	Total Potash.	Calcium carbonate.[b]
				Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
A.....	Wells.....	Clay loam of lacustrine origin. Surface foot.	Neutral.	38.9	8.48	1.55	4.86
B.....	Spring Valley...	Loam from Kansan till plain. Surface foot.	Acid....	23.8	3.97	1.75	.....
C.....	University Farm.	Hempstead silt loam. Surface 6 inches.	Acid....	22.0	48.3	1.78	.....
D.....	Worthington....	Silt loam from late Wisconsin till plain. Surface foot.	Neutral.	31.2	5.95	1.93	.75
E.....	Caledonia.....	Knox silt loam. Surface foot.	Acid....	23.5	2.64	2.25	.....

[a] Organic matter computed from organic carbon using the formula organic carbon × 1.724=organic matter.

[b] Calcium carbonate computed from carbon dioxid.

Soil B was collected from near Spring Valley and is characteristic of the soils formed on the Kansan drift sheet. It was fairly well supplied with organic matter, but strongly acid in reaction owing to heavy precipitation and age of the drift sheet.

Soil C was taken from the surface 6 inches of the Minnesota Experiment Station farm at St. Paul and is classified as Hempstead silt loam (*8, p. 26*). This soil, overlying beds of sand and gravel, is to be regarded as of alluvial origin deposited from slowly running water issuing from the foot of the retreating ice sheet. It shows an acid reaction and is relatively low in total potash.

Soil D, from near Worthington, would be classified as Barnes silt loam and is representative of a large area of well-drained soil developed on the late Wisconsin drift sheet. It is calcareous, as are all of the soils of this type, and being a prairie soil is relatively high in organic matter.

Soil E is a silt loam from the loess near Caledonia in southeastern Minnesota, and would be classified as Knox silt loam. It is poorly supplied with organic matter, is of a strongly acid character, and is high in total potash.

With the exception of sample C, the soils represent composites of 50 individual samples from the surface foot, 10 taken from each of 5 different virgin fields. Soil C was collected from the surface 6 inches of a small field on University Farm that had been in forest plantation for about 30 years.

PREPARATION OF THE SAMPLES

The air-dried soils were reduced with a rubber pestle so as to pass a 2-mm. sieve. Two 1,000-gm. portions of each were weighed out; 10 gm. of pulverized gypsum were sifted over one, placed on a sheet of oilcloth, and the whole was thoroughly mixed. Enough water was sprinkled over each portion to raise the moisture content to about two-thirds the moisture equivalent, after which they were again thoroughly mixed and finally transferred to glass jars of known weight, and enough water added to raise the moisture content to the moisture equivalent. The jars were kept loosely covered with glass plates to prevent excessive evaporation and allowed to remain in an attic storeroom from February 15 until May 15, 1917. At the end of six weeks the jars were weighed and water added to each until the weight was equal to that at the time they were first put aside. The temperature of the storeroom during this period of exposure varied from 10.5° to 18° C. After this the soils were removed from the jars, spread out upon sheets of oilcloth and allowed to become air-dry when they were passed through a 2-mm. sieve and placed in ordinary Mason jars in which they were kept until the analyses could be begun in the following December.

Four hundred gm. of the air-dried soil were weighed out and placed in a 7-liter bottle and treated with 4,000 c. c. of distilled water. At half-hour

intervals for eight hours the contents of the bottles were thoroughly mixed by vigorous shaking. In the case of the pair of soils from each area the two bottles, the one with the treated and the other with the untreated soil, were placed side by side and shaken at the same time, thus insuring the same degree of agitation and extraction. Then they were allowed to stand for 48 hours, or longer if the most of the clay particles had not settled within that time. Then 3,000 c. c. of the supernatant liquid from each was decanted and filtered. Owing to the presence of colloidal clay in the filtered solutions, especially those from the untreated soils, it was necessary to remove this, which was easily accomplished by bringing the solution to the boiling point, adding 0.5 gm. of aluminium chlorid and 5 c. c. of ammonium-hydroxid solution. The flocculated precipitate of aluminium hydroxid on settling removed from suspension the clay particles. After the solutions had been allowed to stand for several minutes, they were passed through ordinary filter papers, giving clear filtrates. The filtrate thus prepared from each soil and representing a definite quantity of this was then analyzed for potash according to the well-known chloroplatinate method, in which the potassium is weighed as potassium chloroplatinate.

TABLE II.—*Effect of gypsum upon amount of water-soluble potash*

Soil.	Weight of soil corresponding to soil extract used for determination.	Determination.	Untreated soil.		Treated soil.		Increase in potash due to gypsum.
			Weight of potassium chloroplatinate.	Percentage of potash.	Weight of potassium chloroplatinate.	Percentage of potash.	
	Gm.		*Gm.*		*Gm.*		*Per cent.*
A	150	1	0.0262	0.00338	0.0327	0.00422	.....
A	150	2	.0269	.00347	.0311	.00401	.....
Average	.....	.....	.0266	.00343	.0319	.00413	0.00070
B	80	1	.0160	.00387	.0260	.00629	.....
B	80	2	.0149	.00361	.0257	.00621	.....
Average	.....	.....	.0155	.00374	.0259	.00626	.00252
C	150	1	.0198	.00255	.0309	.00399	.....
C	150	2	.0196	.00252	.0297	.00383	.....
Average	.....	.....	.0197	.00254	.0303	.00391	.00137
D	80	1	.0098	.00237	.0208	.00503	.....
D	80	2	.0112	.00271	.0178	.00431	.....
Average	.....	.....	.0105	.00254	.0193	.00467	.00213
E	80	1	.0180	.00436	.0362	.00876	.....
E	80	2	.0200	.00489	.0336	.00813	.....
Average	.....	.....	.0190	.00462	.0349	.00845	.00383

In Table II are reported the amounts of soil equivalent to the solution used for each determination, the weight of the potassium chloroplatinate (K_2PtCl_6), and the percentage of the potassium computed as potash (K_2O). Duplicate determinations are reported to show the degree of concordance. The amount of chloroplatinate in each determination was so large as to eliminate the large experimental errors of weighing that occur when only small amounts of soil are employed, while the duplicates in all cases are closely concordant. A blank determination was made with the same kind and quantities of reagents as were used in the actual analyses and the proper corrections made; the gypsum was analyzed and was found to be quite free of potash.

In the case of each soil there is shown a marked increase, due to the addition of the gypsum. The greatest increase was found in the case of soil E in which the gain amounted to over 80 per cent and the least in the case of soil A 20 per cent. While the amount of gypsum employed in the experiment, 1 per cent, equivalent to 10 tons per acre, was much larger than is used in field practice, it would be surprising, in view of the results obtained, if a light application did not cause an appreciable increase in the water-soluble potash.

SUMMARY

Various Minnesota soils when mixed with 1 per cent of gypsum, raised to a point approximating the moisture equivalent, and kept in this condition for three months showed marked increases in the content of water-soluble potash.

The results in previously reported experiments by various investigators in which the action of gypsum has not been found to cause such an increase may be due to the conditions of contact between the soil and gypsum that they have employed being unlike those that obtain in the field.

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